RECLAMATION OF A MIDWEST BROWNFIELD SITE USING AGRONOMIC AND TURF SPECIES

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Abstract

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Plant species were assessed for recolonization of a brownfield in Muncie, IN. In a greenhouse study, soil was seeded to perennial ryegrass (*Lolium perenne*), red clover (*Trifolium pratense*) and sunflower (*Helianthus annuus*). Selected pots were amended with leaf compost and mycorrhizal fungi. Soil and plant tissue were analyzed after 30 and 90 days. Ryegrass and compost were studied at the brownfield site. In the greenhouse, red clover was capable of concentrating the greatest quantity of Cd, Cu, Cr, Ni, and Pb in above-ground biomass (all soil treatments combined). Compost + mycorrhizal fungi treatment resulted in highest Cd, Cu, and Zn plant concentrations (all plant treatments combined). Compost resulted in the highest tissue Cr and Ni concentrations. The reported study demonstrates that this brownfield is capable of being revegetated by turf and legume species. Each infertile and/or toxic site must be assessed for revegetation species on a case-by-case basis.

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INTRODUCTION

Thousands of brownfield sites occur throughout the United States. A brownfield is defined as, "abandoned, idled, or underused industrial and commercial sites where expansion or redevelopment is complicated by real or perceived environmental contamination" (Edwards, 2009). Such sites are often infertile and may be contaminated with heavy metals or other toxic substances. The degree of contamination may pose a public health threat and is, at a minimum, detrimental to plant growth.

Brownfields tend to predominate in old inner city and in low-income areas. Businesses sometimes depart from these communities while buildings are left to be vandalized, degrade over time, and/or release contaminants (e.g., lead-based paint). Potentially toxic components on-site may migrate to the water table, volatilize to the surrounding air, persist in soil, and be taken up by local flora, thus introducing toxins into the food chain (Essoka, 2010).

The cleanup of brownfields can be carried out through several techniques including excavation, surfactant-enhanced aquifer remediation, pump-and-treat, soil vapor extraction, in situ oxidation, bioremediation, and phytoremediation through revegetation (Baker et. al., 1994). Phytoremediation employs the use of vegetation to remove and/or immobilize soil contaminants. A number of plants have been found effective for removal of heavy metals from soil. Revegetation is considered a low-cost and effective treatment for many brownfields. Revegetation is essential in order to prevent erosion by the action of water or wind, limit runoff of metallic effluents, and to create an aesthetically pleasing site. In many cases, however, revegetation efforts at brownfields are hindered by poor soil quality (e.g., low fertility, low organic matter content, poor soil structure).

When brownfields are remediated, several long-term benefits occur such as community revitalization, increased real estate values, improved aesthetic, and the possibility of profitable businesses returning to and adding revenue to the community (Hong-Bo et al., 2010). Other benefits include removal of toxins from soil and groundwater, and enhanced public health (Baker et al., 1994).

The former Car Doctor's Salvage Yard in Muncie, IN was listed in the Center for Public Environmental Oversight's Brownfield Archive in 2008 as part of a Phase I Site Assessment report. Research on soils of this site is the focus of the current thesis.

LITERATURE REVIEW

Many commercial and industrial sites in industrialized nations are contaminated with potentially toxic metals such as Cd, Cr, Cu, Ni, Pb and Zn. The estimated yearly worldwide release of metals (in metric tons) over the last two to three decades has been: 22,000 t of Cd, 939,000 t of Cu, 783,000 t of Pb, and 1,350,000 t of Zinc (Padmavathiamma and Li, 2007; Singh et al., 2003). Several of these metals, when concentrated in soil, pose a threat to public health and the environment. Metals can leach through the soil profile; be carried away in surface runoff; accumulate in plants to the point at which they become phytotoxic; and can spread throughout the food chain. Metal contamination of soils by anthropogenic inputs has been discovered in industrial areas, mine sites, urban areas, near metal smelters, and in waste disposal areas and along roadsides (Pichtel, 2007). Subsequent heavy metal contamination from these activities must be removed from or immobilized in soils because they cannot be chemically degraded (Isoyama et al., 2007). In order to carry out effective treatment, it is essential to understand the chemical behavior of metals in soil.

Behavior of Selected Metals in Soil

Cadmium

At contaminated sites, cadmium exists primarily as the Cd^{2+} ion, Cd-CN^- complexes, or $Cd(OH)_2$, depending on pH and waste processing prior to disposal (U.S. EPA., 1995). During weathering Cd readily solubilizes. It bonds to soil organic matter, clays and other colloids; however, Cd may be exchanged by other cations in soil. The most significant environmental factors which control Cd ion mobility are pH and oxidation-reduction potential. Cadmium is most mobile in soils at pH values $\leq$ 5.0, whereas in neutral and alkaline soil Cd is rather immobile. At pH values < 8 it exists as the Cd^{2+} ion. In addition, it may form complex ions such as $CdCl^+$, $CdHCO_3^+$, $CdCl_4^{2-}$, $Cd(OH)_3^-$, and

$Cd(OH)_4^{2-}$ and organic chelates (Pichtel, 2007). Cadmium can be taken up by plants; however, it is phytotoxic to many species.

Chromium

Chromium exhibits variable oxidation states (from 0 to +6). The Cr^{6+} ion, common in industrial uses and which is both mutagenic and toxic, forms anions such as chromate (CrO_4^{2}-), bichromate ($HCrO_4^{-}$), or dichromate ($Cr_2O_7^{2-}$). These species remain soluble in soils and sediments; thus, the risk of groundwater contamination is significant (Nivas et al., 1996). The Cr^{3+} ion is rather water-soluble as compared to the Cr^{6+} ion which is less mobile; it is therefore, of less concern as a contaminant of groundwater and soil (Nivas et al., 1996). Certain plant species have been shown to reduce the highly toxic Cr^{6+} to the less toxic Cr^{3+} ion.

The environmental behavior of Cr depends upon soil pH, oxidation state, mineralogical properties and presence of organic matter. Chromium behavior is governed strongly by both soil pH and redox potential (James and Bartlett, 1983; Bartlett and Kimble, 1976a; 1976b). The adsorption of chromate by soils is rather poorly understood. The CrO_4^{2-} anion may be adsorbed to Fe and Al oxides and with other positively-charged colloids.

Copper

Copper is used in electrical wiring, plumbing, pigments, medical devices, casting, wood treatment, and for alloying with bronze, silver, and brass (Altaher, 2001). Copper contamination can occur through mining activities, phosphate fertilizer production, fugitive emissions from manufacturing facilities, and through natural phenomena such as forest fires (Lenntech, 2013).

Copper exists in soil as $CuFeS_2$, Cu_2O, CuO, $CuSO_4$, and as CuS (Chang, 2010). It can be bound to soil in several ways: to the exchangeable complex; and to carbonates, silicates, organic matter (humus), and oxides (Fe and Mn). Small proportions may also be soluble in water. The bioavailability and mobility of Cu depends on the presence of these forms and other soil parameters such as the amount of Mn and Fe, and carbonate content. Decreased soil pH increases mobility (optimal at 6.24). Copper ions become highly complexed with organic matter (Altaher, 2001).

Nickel

Nickel is used for alloys, electroplating, batteries, coins, industrial plumbing, spark plugs, machinery parts, stainless-steel, nickel-chrome resistance wires, and catalysts (US EPA, 1986). Land application of industrial wastes and biosolids has been shown to be a source of Ni to soils and to plants.

Nickel enrichment of soils greatly influences its concentrations in plants. Nickel is readily mobile in plants; several grains have been found to contain elevated Ni concentrations (Kabata-Pendias, 2001). The soluble, adsorbed (exchangeable) and organically-bound fractions of soil Ni are the forms most available to plants (Pichtel and Anderson, 1998). Both plant and soil factors affect Ni uptake by plants, but the most significant factor is soil pH (Kabata-Pendias, 2001). Nickel is readily taken up and until certain concentrations in tissues are reached, absorption is positively correlated with soil Ni concentration.

Lead

Lead (Pb) is released to the biosphere primarily from metal smelting and processing, secondary metals production, Pb battery manufacturing, pigment and chemical manufacturing, and disposal of Pb-containing waste. Specific Pb sources include industrial waste and construction debris buried in landfills, lead-based paint, high explosive burn sites, firing ranges, and disposal and storage of lead acid batteries (Pichtel, 2005; Seth and Singh, 2011).

Lead is among the least mobile among the heavy metals. Contamination of soils with Pb is mainly an irreversible and, therefore, a cumulative process in surface soils (Smith et al., 1995). Negligible Pb is transported into surface water or groundwater. The fate of Pb is affected by adsorption, ion exchange, precipitation, and complexation to organic matter. In soils, Pb forms stable complexes with both inorganic (e.g., Cl^- , CO_3^{2}-) and organic (e.g., humic and fulvic acid) ligands (Bodek et. al., 1988). A limited number of plants are known to uptake soil Pb; however, the majority of plants tend to exclude it.

Zinc

Zinc is released to the atmosphere as fumes and dust through fuel and coal combustion, smelter and metalworking industries, and municipal solid waste incineration. The most common and mobile Zn form in soils is Zn^{2+}. Those factors most significant in controlling Zn mobility are pH; and presence of hydrous oxides, clay minerals, and soil organic matter. Zn forms in soil may be altered by adding soil amendments (Pichtel and Bradway, 2008). As compared to other heavy metals, Zn is readily soluble, especially in acid light mineral soils.

Zinc is a trace element essential for growth of plants and animals and is not considered to be highly phytotoxic. Soils that have been amended with municipal solid waste (pH 6.8) contain mobile Zn which can readily be taken up by plants (Pichtel, 2007). Many plant species have been shown to accumulate significant quantities without showing signs of toxicity; however, Zn-Cd interactions are common and Cd is highly toxic to plants.

Conventional Methods of Soil Remediation

Methods of treating metal-contaminated sites have, in recent years, involved one or more of the following: solidification/stabilization, electrodialytic (EDR) or electrokinetic (EKR) removal, extraction through soil washing (ex-situ)/flushing (in-situ), containment, and soil excavation/disposal. Most of these methods are costly due to the equipment and specialized operators required (Mukhopadhyay and Maiti, 2010). These technologies also have the potential to remove or destroy all biological activity, soil structure, and micro- and macronutrients during the remediation process (Baker et al.,1994), all of which are essential to soil health and can determine the responsiveness of the affected soil to remediation. Extractive processes have an advantage over immobilization processes in that contamination can be permanently removed, thereby reducing future liability for the site owner.

Soil washing (ex-situ) is an extractive process, in which the affected soil is mixed with a washing solution. Contaminants become desorbed from sediment surfaces and complex matrices, and are suspended in an aqueous phase (Fortin et al., 2003). This is followed by separating contaminants from the washing solution (Fortin et al., 2003; Isoyama et al., 2007). Soil washing techniques are used to remove halogenated solvents, aromatics, PCBs, fuel oils, chlorinated phenols, pesticides, and soluble metals from soil (Fortin et al., 2003).

Soil flushing (in-situ) involves applying water, sometimes containing additional treatment compounds, to the soil surface or injecting it directly into groundwater in order to solubilize and recover contaminants (U.S. EPA, 2012). Soil washing techniques using solvents and chelating agents are associated with several negative effects on soil characteristics (e.g., loss of soil nutrients, loss of structure); however, this method has proven to be highly effective at removing pollutants.

Electrodialytic (EDR) or electrokinetic (EKR) techniques involve placing electrodes within soil and applying a direct current. Soluble heavy metals, typically occurring as cations, are drawn through soil to the electrodes through electro-osmosis, electrophoresis, and/or electro-migration (depending on the metal) and subsequently are recovered (Cang et al., 2011).

Immobilization (solidification/stabilization) of heavy metals is considered for areas where other techniques are not feasible due to expense, or on sites where permanently immobilizing the metal ensures that it would not enter the groundwater or the food chain. Isoyama et al. (2007) found that soil Pb was immobilized by addition of calcite mixed with allophanic, smectite, or kaolinitic soil, which both increased pH and sorbed soil Pb (Isoyama et al., 2007). Immobilization may involve reagents as simple as Portland cement, which is mixed into soil either at the surface by front-end loaders, or to greater soil depths using multiple augers (Pichtel, 2007). Once the soil-cement mixture has set, the contaminants are chemically stabilized and/or locked into a solid matrix.

In recent decades, green plants have been studied extensively for redevelopment and remediation of metal-contaminated sites (Mukhopadhyay and Maiti, 2010). Research has involved simple revegetation, where suitable plant species are used to create green belts and protect soil from erosion. Additionally, plants have been studied for their ability to remove soil metals for eventual treatment (“phytormediation”).

Revegetation of Infertile Soils

Revegetation of a disturbed or contaminated site is feasible with the proper plant species and amendment(s), even in heavily metal-contaminated and low organic matter sites. Ryegrass (*Lolium perenne*) establishes and grows quickly to provide effective surface cover which increases organic matter content; furthermore, it provides erosion control, keeps soil from drying, and suppresses annual weeds, at least during the first year of growth (Ye et al., 2000; Zelnik et al., 2010).

Plants of the species *Trifolium* (e.g., red clover, *Trifolium pretense*) are used to colonize metal-contaminated soils by increasing soil N content. Nurse grasses (e.g., *Lolium* species) and legumes (*Trifolium* species) are competitive ruderals, meaning they adapt to conditions where competition is moderate (Zelnik et al., 2010).

Tap-rooted legumes increase the formation of air-filled pores in soils having poor structure through "biological drilling or ploughing" (Hall et al., 2011). Pastures of the legume Caribbean stylo (*Stylosanthes hamata*) and Alyce clover (*Alysicarpus vaginalis)* have been shown to increase macropores as compared to native grassland (Hall et al., 2011). Plant roots in general improve soil structure and aeration by enlarging pore spaces, increasing water infiltration, and stabilizing soil particles via enmeshing in their fibrous root systems which release soil-aggregating compounds (Hall et al., 201; Garbeva et al., 2004).

Use of Soil Amendments

At many disturbed sites, soil quality is so poor that many common agronomic and turf species can neither establish nor grow. Soils may be deficient in several macronutrients, enriched with toxic elements, possess poor structure, and lack organic matter (Jacob et al., 2007). In such cases it is necessary to amend the soil to improve adverse conditions prior to attempting to establish cover crops.

Inorganic nitrogen fertilizer imparts a positive impact on bacterial and actinomycete communities as well as plant communities (Garbeva et al., 2004). Low-N amendments resulted in "high-density/low frequency" vegetative cover; in other words, plots had high plant coverage but plants were stunted, and a diversity of undesirable forb species and weeds proliferated. High-N amendments resulted in a frequency of 75-90% desired plants. The low-N plots promoted the establishment of weeds and other invasives due to their ability to tolerate relatively infertile conditions (Garbeva et al., 2004).

Seeding of *Lolium* species resulted in high plant coverage on highly toxic Zn/Pb mine tailings with addition of mushroom compost as a soil barrier (Ye et al., 2000). Plants may absorb high quantities of metals when grown with barrier layers and have been shown to accumulate greater quantities with the presence of a thicker barrier layer, whether as coal ash, fly ash, swine manure, or mushroom compost (Ye et al., 2000).

Lange et al. (2011) found that humus substitution products such as Novihum™ (an artificial humus soil conditioner) and Stocksorb™ (an organic synthetic polymer) increased the water-holding capacity of uranium tailings when grown to sessile oak (*Quercus petraea*). These products are classified as long-lasting fertilizers (LLF) because they release N over extended periods. The amendment increased photosynthetic efficiency by 49% over control plots; additionally, trees growing on amended plots increased in diameter by 129% after four growing seasons as compared to control plots. Plots amended with New Red™ Rotliegendes mineral soil material (a highly mineral-enriched soil from Germany) on uranium-contaminated soil supported a variety of trees including willows, birches, and aspens, thus indicating a low-cost remediation solution.

The use of compost (e.g., solid waste, sewage, agricultural compost) is a recent development for aiding plant establishment on brownfield sites (Sparke et al., 2011). Compost imparts chemical, physical, biological, and plant-colonizing effects on soil; thus, it is recommended for long-term restoration of infertile soils (Tejada and Gonzalez, 2008; Sparke et al., 2011). Composts are rich in organic matter, which improve adverse physical properties such as soil compaction, and low nutrient and microbial levels. Compared to animal manure, mature compost does not contain detrimental organisms that may inhibit seed germination and plant growth (Sparke et al., 2011).

Various amendments were applied to waste rock piles in the Silver Dollar Mine Site in northern Idaho (McGeehan, 2009). The soil was a sandy loam with pH = 8.3. Amendments included biosolids, EKO compost (composed of community-collected organic materials and biosolids from the local wastewater treatment plant) and urea plus log yard waste. Additional amendments included Kiwi Power™ (non-plant food which improves soil physical properties), Glacier Gold™ (nutrient-rich topsoil), and control plots. Nitrogen availability from compost positively affected density, frequency, and diversity of grass and forb species such as clover and yarrow. Nitrogen concentrations in runoff were also reduced.

Aschenbach et al. (2012) compared seed germination and biomass production of two grass species, little bluestem (*Schizachyrium scoparium*) and Indiangrass (*Sorghastrum nutans*) to two legume species, sundial lupine (*Lupinus perennis*) and Illinois bundleflower (*Desmanthus illinoensis*) in a greenhouse study on spoil from a sand mine in the Great Lakes Basin, Michigan. Spoil was amended with sphagnum peat moss and inorganic fertilizer. The bundleflower experienced highest germination (39%). Peat treatments experienced increased seed germination (up to 25%) for all species, compared to an 18% germination rate without amendment. Peat treatment also resulted in a mean 0.028 g biomass/pot while the control produced 0.017 g biomass/pot. Inorganic fertilizer resulted in similar data to those for peat moss. For lupine, biomass production was not significantly affected by application of amendments; the authors promote this species along with Illinois bundleflower as candidates for revegetation of sand mine spoils because they both fix N and are native legumes.

Substrate conditions, plant uptake, and diversity were examined by Courtney et al. (2009) to measure the success of revegetation of three bauxite waste disposal sites. The study took place on weathered “red mud” which was amended with process sand, spent mushroom compost, and NPK fertilizer. One site was amended with a process sand/gypsum mixture. After two years, 47 species were growing on the residue (compared with the original six species that were planted). Red clover (*Trifolium pratense*) and yorkshire-fog *(Holcus lanatus*) were the dominant species and comprised part of the original seedling application. Perennial ryegrass (*Lolium perenne*) and *H. lanatus* showed signs of foliar nutrient deficiency, and P intake was especially limited by the high adsoprtion capacity of the bauxite. Soil K and N levels were sufficient, and nutrient and organic matter build-up in the soil was documented. Long-term management is still considered necessary due to low P uptake.

Arbuscular Mycorrhizal Fungi and Site Revegetation

Arbuscular mycorrhizal fungi (AMF) are an abundant microbial community in soils which form symbiotic relationships with plant roots to enhance plant acquisition of water and mineral nutrients, especially immobile elements such as S, Ca, K, Fe, Mg, Mn, and N. (Linderman, 1988). AMF have symbiotic associations with an estimated 80-90% of vascular plants and some non-vascular plants such as mosses (Alori and Fawole, 2012). In addition, AMF contributes to soil aggregation and helps plants grow in soils of low fertility, high risk of erosion, low pH, high phosphorus fixation, low organic matter content, metal toxicity, and/or limited biodiversity (Alori and Fawole, 2012). AMF promotes seedling establishment by integrating emerging seedlings into extensive hyphal networks and by supplying nutrients to the seedlings (van der Heijden, 2004). AMF, therefore, act as a symbiotic support system that promotes plant establishment. AMF also helps reduce stress to plants growing in soils with high salt content, metal-enriched mine spoils, and landfills; furthermore, they have been demonstrated to reduce the occurrence of plant pathogens and disease (Alori and Fawole, 2012; Garbeva et al., 2004).

Significant enhancement of activity of actimomycetes, nitrogen-fixing, and phosphate-solubilizing bacteria in cooperation with AMF have been observed in the rhizosphere (Linderman, 1988). Alori and Fawole (2012) conclude that a positive relationship exists between AMF biomass and metal concentrations in soil. The ability of AMF to treat metal-enriched soil may depend on ensuring sufficient AMF biomass, i.e., it must occur in proportion to soil metal concentrations (Alori and Fawole, 2012).

Phytoremediation

Phytoremediation is defined as the engineered use of green plants for removing and/or decomposing contaminants at an affected site (Mukhopadhyay and Maiti, 2010). Of all available remediation technologies, phytoremediation imparts the most positive effect on soil properties including enhancing biological activity and diversity, maintaining soil aggregation, and enhancing macro- and micronutrient concentrations (Garbeva, et al., 2004). Furthermore, the method operates in-situ (i.e., in-place – there is no extensive earthmoving). Phytoremediation is most effective when both the biological mechanisms of the plants are understood, and when the engineering, physiological, and molecular factors of the entire process are carefully planned and considered (Padmavathiamma and Li, 2007). Phytoremediation may be divided into several modes, depending on how the plant is being utilized: phytovolatization (the plant volatilizes the contaminant); phytostabilization (the plant decreases the bioavailability of the contaminant); phytodegradation (the plant takes up and decomposes the contaminant), rhizofiltration (the plant is used to decontaminate soil water); and phytoextraction (the plant concentrates the contaminants its biomass) (Chaney et al., 2000; Salt et al., 1996).

Phytoextraction

For so-called phytoextraction of soil metals to be effective, plants must be capable of absorbing high levels of the metal(s) of interest (Padmavathiamma and Li, 2007). Additionally, however, the plant must possess the ability to reduce the toxicity of the soil metal(s) either in the root zone or within its tissue (Salt et al., 1996). Hyperaccumulating plants are defined as species which can accumulate >1% of their shoot dry mass as metals (Chaney et al., 2000; Salt et al., 1996). Brooks and Reeves (1977) first used the term "hyperaccumulator", which they assigned specifically to Ni-accumulating plants that take up >1000 mg/kg of Ni; however, the definition also applies to plants that accumulate the same quantities of Co, Cu, and Zn (Chaney et al., 2000). A small percentage of hyperaccumulating plants are able to accumulate >1% of several metals simultaneously (Salt et al., 1996).

The original discovery of so-called metal hyperaccumulators is unclear, because the knowledge that plants are capable of absorbing metals was understood for centuries (Padmavathiamma and Li, 2007). The German botanist Baumann confirmed in 1885 that pennycress (*Thlaspi calaminare*) was able to accumulate up to 17% ZnO in shoot biomass (Chaney et al., 2000). Baumann later determined that Alpine pennycress (*T. caerulescens*) growing wild on Zn-contaminated soils contained about 1-1.7% Zn in dried leaves (Salt et al., 1998). During this time, plants known to accumulate metals were termed bioindicator plants, and this field of science was applied toward the identification of promising metal mining sites. Bioindicator plants proved to be important for the discovery of uranium (U) ores in the USSR and the United States (Chaney et al., 2000).

In South Dakota in 1935 it was discovered that Se was accumulating in certain forage crops, which was eventually identified as the cause of a debilitating disease in range animals (Salt et al., 1996). Elsewhere, Mo poisoning (via hyperaccumulation by forage crops) was discovered to affect ruminant animals (Chaney et al., 2000). Around the same time it was found that dried leaves of Brassiceceae (*Alyssum bertolonii)* contained about 1% dry biomass as Ni (Salt et al., 1996). Botanists found that this concentration was 100-1000 times higher than that of other plants growing nearby. The Fabaceae family, which is also known as the legume, bean, or pea family, was the first family of plants, along with the Brassicaceae, to become distinguished as hyperaccumulators (Salt et al., 1996).

It was not until the early 1990s that hyperaccumulator technology was realized as a viable use of plants to degrade, immobilize, or extract anthropogenic contamination from soil and/or water (Mukhopadhyay and Maiti, 2010). The first field experiments employing continuous phytoextraction were conducted by Chin et al. (1992) using Alpine pennycress (*T. caerulescens*) in the US and at Rothamsted Research Station the UK. Baker et al. (1991) conducted the first field experiments on the possible phytoextraction of Cd and Zn (Padmavathiamma and Li, 2007). These studies showed that increasing soil acidity resulted in increased uptake of Cd and Zn. Chin et al. (1992) also discovered that Alpine pennycress provided more complete ground coverage when fertilizer was applied and plant competition was controlled by weeding (Chaney et al., 2000). Phosphorous was also confirmed to decrease the ability of the plant to uptake Pb. Soon after, Asteraceae (*Berkheya coddii*), of northeastern Transvaal, South Africa, which produced a higher biomass than most of the other hyperaccumulators classified at the time, was discovered to accumulate as much as 3.7% of dry shoot biomass as Ni (Salt et al., 1996).

Currently, 45 plant families are known to contain metal hyperaccumulator species, and 400 metal hyperaccumulator taxa have been categorized since 2006 (Salt, 2006). These species are found growing on metalliferous soil and are, therefore, referred to as “metallophytes” whether they hyperaccumulate metals or not (Schat et al., 2000). Each metallophytic species demonstrates differing patterns of uptake, tolerance, and root-to-shoot transfer of specific metals (Schat et al., 2000). It is still not completely clear just why hyperaccumulators take up large amounts of metallic elements or what their role is in an ecosystem, but the most accepted theory to date has been the “elemental defense” theory, in which plants passively uptake toxic metal ions in order to avoid attack by fungi and insects (Boyd, 2012; Salt et al., 1996, Schat et al., 2000). Nickel is known to protect *Streptanthus polygaloides* against bacterial and fungal disease, and also protects *T. montanum* and *S. polygaloides* from insect infestation (Salt et al., 1996).

Recent research has examined the use of plants as an extractive tool for metal-contaminated soils (Mills et al., 2006; Tie et. al., 2006; Datta and Sarkar, 2005; Pichtel et al., 2000). Phytoextraction has received extensive scientific attention over the past two decades, and its development becomes more attractive when compared to the cost of complete soil removal, which is estimated at about $1 million/acre (Salt et al., 1996), or up to $500/ton, not including the cost of transport and subsequent landfill monitoring (Padmavathiamma and Li, 2007).

For site revegetation and subsequent phytoremediation to succeed, the degree of plant tolerance to metallic contaminants must be determined (Schat et al., 2000). Plant uptake of metals and/or biomass production serve as a measure of a plants’ suitability for treating a site. A number of researchers have documented success with plant extraction of heavy metals (Wu et al., 2006; Li et al., 2005; Wilde et al., 2005). Long-term, i.e., continuous phytoextraction relies on the plant’s natural physiological processes and genetic capabilities to translocate and accumulate heavy metals, generally requiring several growing seasons (Salt et al., 1996).

Several disadvantages confront researchers in the use of hyperaccumulating plants for site remediation: the most effective plants generally produce low biomass, have slow growth rates, and few plants are documented which take up >1% (w/w) of Cd, Pb, U and As (Salt et al., 1996; Linacre et al., 2003). The development of transgenic plants may be a solution to some of the disadvantages of hyperaccumulators. The possibility of transferring hyperaccumulating genes into large plants has been explored, particularly within the last decade. The technology involves transferring genes from a small hyperaccumulating species of an especially harmful element like Cs into larger plants which would normally die under low Cs doses (Chaney et al., 2000).

In order for transgenic plants to be used on a widespread scale, a complete understanding of the biological processes occurring in non-genetically modified plant species must be determined (Hur et al., 2011; Linacre et al., 2003; Pilon-Smits, 2002). Characteristics to be studied through genetic testing of plants include plant height, biomass yield into maturity, rate of re-growth after stem cutting, and the ensured inheritance of metal uptake genes (Chaney et al., 2000). This is especially important considering the possible, unknown risks that transgenic plants may pose in field experiments (Salt et al., 1996; Linacre et al., 2003). Those who argue against genetic engineering cite biosafety issues of allowing engineered plants to become invasive species (which may also contain toxins) that could enter the food chain (Linacre et al., 2003). Schat et al. (2000) states that commercial application of transferring hyperaccumulator genes of high-biomass crop species is still a far-off general practice. This is because many studies suggest that hyperaccumulation is an intricate phenomenon where root metal absorption, tolerance to toxicity, and subsequent metal transport from root to shoot are determined by separate processes and genes (Mukhopadhyay and Maiti, 2010; Schat et al., 2000).

A former zinc mine site was grown to wild type and genetically modified (GM) poplars (*Populus deltoides*) for three years. Maximum metal extraction was 1.6-fold greater for the GM poplars (Hur et al., 2011). Debate has ensued as to whether genetically modified plants negatively alter microbial communities; in the Hur et al. (2011) study, however, the GM poplars showed a more rapid shift in soil microbial communities because of accelerated pH change in the rhizosphere which also resulted in more active metal uptake (Hur et al., 2011).

Chelate-Assisted Phytoextraction

Chelate-assisted phytoextraction may be considered if an affected site is highly saturated with toxic metals and/or the site poses an immediate threat to nearby populations and ecosystems (Salt et al., 1996). A chelating agent is defined as a large, multi-dentate organic compound saturated with negative charges (Pichtel, 2007). Some chelating agents are synthetic (e.g., EDTA), while others are naturally-occurring (humic compounds) (Seth and Singh, 2011). Chelate-assistated phytoextraction is used commercially because it greatly increases plant uptake of metals, and can enhance uptake of several different metal species simultaneously (Bricker et al., 2001; Salt et al., 1996). Ethylenediaminetetraacetic (EDTA) is a common chelate used on contaminated soil, especially when remediating Pb-contaminated sites (Seth and Singh, 2011). Ethyleneglycol-tetraacetic acid (EGTA) is generally used for Cd contamination while citrate has been used to treat U contamination (Salt et al., 1996).

Plant extraction of soil metals is reliant on the production of chelating agents secreted from the plant rhizosphere (Mukhopadhyay and Maiti, 2010). Soil colloids naturally bind and complex with metals, and plants have evolved strategies to desorb these metals, thus increasing their availability (Salt et al., 1996). One such plant-produced chelating compound, a phytosiderophore, is a complex molecule synthesized and secreted by roots to enhance uptake of Cu, Fe, Zn, and Mn (Fig. 1) (Salt et al., 1996). After the siderophore binds to the metal ion in the soil, a specific membrane transporter aids in carrying the metal-siderophore complex into the plant. The complex then becomes stored in root cells within vacuoles or transported to the shoot via the xylem (Salt et al., 1996).

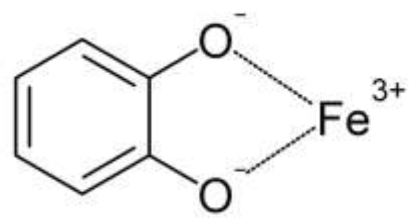

Fig. 1: A catecholate (phenolate) – iron complex; catecholates are one of the major groups of siderophores which are known to be the strongest Fe^{3+} binding compounds. Source: http://en.wikipedia.org/wiki/Siderophore.

Transpiration facilitates the movement of metal ions through xylem vessels, creating mass flow of the ions into the shoot (Salt et al., 1996). Metal-chelate complexes such as Cd-citrate, which are non-cationic, are transported more easily through the plant than a Cd^{2+} ion alone. Xylem cell walls have a high cation exchange capacity and therefore reduce movement of cationic Cd while Cd-citrate flows easily (Salt et al., 1996). Plant-produced citrate has been shown to chelate Fe^{2+} and Zn^{2+}, and Cu^{2+} becomes complexed with amino acids, including asparagine and histidine. Plants of the genus *Alyssum* contain the amino acid histidine which binds Ni while accumulating in its xylem. Iron, Mn, Ni, Co, and Zn also bind with nicotianamine (Salt et al., 1996). Using natural chelates during site remediation is considered beneficial over use of synthetic chelates by reducing costs and potential harm to the local environment.

Important metal-transport peptides in plants are termed metallothioneins (MT) and phytochelatins (PC) (Goldsbrough, 2000; Schat et al., 2000). Both are strongly attracted to metal ions. Metallothioneins have a low molecular weight and are rich in cysteine polypeptides (Goldsbrough, 2000; Salt et al., 1996). Phytates also form insoluble complexes with Fe, Zn, and Cu, and even though they are not considered chelators, they occur naturally in plants and may be considered as a detoxification mechanism for Zn (Goldsbrough, 2000). Phytochelatins are similar to metallothioneins in that they are of low molecular weight and are rich in cysteine peptides; however, phytochelatins also bind strongly to Cd as high- or low- molecular weight complexes and are important for plant resistance to the toxic effects of Cd (Goldsbrough, 2000; Salt et al., 1996). Other PC-metal complexes that have been extracted from plants include those with Ag and Cu (Goldsbrough, 2000).

There is evidence that plants may also secrete protons in order to acidify the rhizosphere because metals become more bioavailable at low pH values (Mukhopadhyay and Maiti, 2010; Pichtel, 2007). It has been suggested that organic acids may play a role in the movement of Cd through the shoot (Seth et al., 2011). Each of the discussed compounds and mechanisms are highly dependent on plant type and plant nutritional balance (Salt et al., 1996).

Practical Considerations in Phytoremediation

Significant benefits of soil treatment via phytoremediation include: plants stabilize soil through their roots, increase water infiltration, reduce erosion, and reduce surface water runoff (Garbeva et al., 2004). One of the key advantages of phytoremediation over other technologies, however, is significantly lower cost. "The usage of plants in… bioremediation is a favorable option, with minimal negative effects on the soil… (phytoremediation has) the advantage of removing metals from soil," and in comparison to soil washing, "(it) is a 50-80% cheaper method comparing with classical methods" (Elekes and Busuioc, 2011). Chaney et al. (2000) estimates the total cost of conventional remediation at $8-24 million per hectare to 1 m depth. This cost involves the subsequent replenishment of the site with clean soil and the disposal of the toxic soil to a landfill.

In order to remove contaminant metals from a site it is necessary to harvest the metal-enriched plant tissue. Options for the management of metal-laden tissue include drying or ashing followed by recycling the metals, or disposing as hazardous waste (Baker et al., 1994). The former option is also known as "phytomining" where the plants are pyrolyzed and the ash is sold as metal concentrate (Chaney et al., 2000). This method helps to reduce cost or even accrue a profit, unlike traditional remediation methods. Pyrolysis of biomass has even been proposed by the U.S. Department of Energy as a possible source of energy. Pyrolysis is especially cost-effective and profitable when ash from plants contains 10-40% Ni, Cu, Co, or Zn (Chaney et al., 2000).

The development of a non-intrusive greenbelt area during the remediation period is more aesthetically pleasing than other alternatives using machinery, hazardous reagents, and excavation (Smith et al., 2011). Plants also reduce the dispersal of metal-contaminated dust during the remediation activity.

Phytoremediation is not without its drawbacks, however. The process may be slow and is therefore not suitable at sites posing an immediate public health or environmental threat. It generally takes several successive growing seasons because most plants that are used for this method accumulate <1% dry metal weight in their biomass (Jiang et al., 2010). There is additionally a potential for dispersal of metal-laden plant tissue. Phytoremediation is not a suitable option for sites where contamination is located at great depths. It is optimal at sites where contamination is limited to the uppermost layer of soil. Many metals (for example, Pb) migrate little from their point of deposition. In cases where metals are deposited at or near the surface, such as by atmospheric deposition or shallow disposal, phytoremediation may be a viable option. Although limited to plant root zones, this may encompass most of the contamination found at certain brownfield sites (Kovalick and Kingscott, 1996).

Choosing a Remediation Strategy

In choosing a remediation strategy for a metal-affected site, the end-use for the site must be considered. Factors to address include exposure routes of metals, and location of populations and sensitive environmental receptors (Essoka, 2010). If Pb is the principal contaminant, options such as no action, containment, or off-site disposal can be considered due to the limited solubility of Pb in water and soil. Conditions that rule out these options include proximity to populations, the presence of highly permeable soil, acidic soil reaction, or large quantities of toxic contaminants (Royer et al., 1992).

The ultimate goal of remediation is to remove contaminants; another key goal is to “restore the capacity of the soil to perform or function according to its potential” (Jiang et al., 2010). This is why indicators of soil quality must be assessed and monitored during and after remediation, which include keeping track of microbial diversity of the soil, physical and chemical changes in plants, and ensuring that remediation efforts are not destroying soil structure (Garbeva et al., 2004).

Little is known regarding the revegetation and/or treatment of metalliferous soils in brownfields. Such soils may additionally be affected by infertility, poor drainage, low organic matter content, and limited populations of indigenous microorganisms that cycle nutrients. Numerous studies have been carried out using municipal and industrial wastes as soil substitutes on drastically disturbed and/or contaminated soils (Pichtel and Bradway, 2007; Halofsky and McCormick, 2005; Pichtel and Dick, 1991a; 1991b). Little has been documented, however, concerning use of soil amendments and mycorrhizal fungi on plant response at brownfields.

Section II. Technical Report

Objectives

The present study was established to determine the feasibility of revegetation of the former Car Doctors brownfield site in Muncie, IN. Additionally, efforts were devoted toward both monitoring and enhancing the removal of soil metals.

Specifically, the objectives were to:

1. compare the efficiency of selected plant species in colonizing a relatively infertile soil;
2. assess the influence of mycorrhizal fungi and leaf composted biomass in improving plant yields at the site; and
3. compare the efficiency of plant species in either removing or stabilizing selected soil metals (Cd, Cr, Cu, Ni, Pb and Zn).

Experimental Methods

The Site

The former Car Doctors is located on Burlington Drive, Muncie, Indiana (LAT/LONG: 40.1881 / 85.3628). The site had been used as a commercial and industrial facility since 1934. From 1934 to 1971 the site was used for a bulk oil and gas storage refinery and several other businesses. From 1976 to 2002 it was used as an automotive salvage yard and has been vacant since 2003. During a site assessment, tires, automotive parts, construction and demolition debris, trailers, and general refuse were identified. There is evidence of unknown materials dumped or buried and also residues from oil and gasoline releases. Some spills are recorded; however, there are concerns regarding gaps in historical data for the site.

Greenhouse Study

A greenhouse experiment assessed metal phytoextraction from soil collected from the site. Soil material was collected from the upper 20 cm and returned to the laboratory where it was air-dried and sieved through a 2-mm mesh sieve. The soil was placed into 48 plastic pots measuring 17 cm diameter by 18 cm tall. Each pot contained 1 kg soil.

Pots were divided into vegetative treatments containing perennial ryegrass, red clover, sunflower or control (no plants). Soil treatments included mycorrhizal fungi; organic compost; both mycorrhizal fungi and compost; or no amendment. Greenhouse temperature was maintained at 80-95^{o}F (29-32^{o}C) and relative humidity at 55-65%. Artificial lights were kept on all pots continuously.

Ryegrass and red clover seeds were applied at 5 ml/pot; ten sunflower seeds were placed within selected pots. Mycorrhizal fungi was applied 2.5 ml per pot, and leaf compost was applied at 72.5 g per pot, equivalent to 0.0725 kg/ha. Compost was supplied from the Ball State University Heath Farm.

Soil was sampled at 30 and 90 days after seeding. Sampling was accomplished by inserting a stainless steel sampling tube into each pot and withdrawing samples. Soil material was air-dried for 48 h at room temperature.

Determination of soil K, Cd, Cr, Cu, Ni, Pb, and Zn was carried out after shaking 5 g of soil with 25 ml of 5 mM diethylenetriaminetriacetic acid (DTPA) on an oscillating shaker (120 min) and measurement using flame atomic absorption spectrophotometry (FAAS) (Perkin-Elmer Aanalyst 2000, Norwalk, CT).

A subsample of soil from the pots was analyzed for particle size distribution using the hydrometer method (Day, 1965). Organic carbon content was determined by loss on ignition at 360^{o}C (Nelson and Sommers, 1982), and pH in a 1:2 (w:v) soil: deionized water slurry. Soils were tested for soluble NO_3 and NH_4^+ by the microplate method (GEN 5 Microplate Spectrophotometer Powerwave X5/X52 by BioTek). The organic compost was also tested for the above parameters.

Above-ground plant tissue was sampled at 30 d after seeding. After 90 d, both plant and root tissue were harvested. Above-ground biomass was cut approx. 1-2 cm above ground surface and placed into paper bags where it was dried for 48 hours at 105^{o} C. After roots were removed from the pots they were rinsed with tap water and then with deionized (DI) water before being oven-dried (48 h at 105^{o} C). A total of 0.55 g tissue, whether above-ground or roots, were mixed with 10 ml of 75-80% concentrated HNO_3, and microwave-digested (MARS microwave digestor, CEM Corp., Matthews, NC). The method used for plant digestion was as follows: vessel temperatures were ramped to 190^{o}C for 15 min with a holding period of 15 min at 800 W. The cooled digestates were diluted with 40 ml DI water and analyzed for Cd, Cr, Cu, Ni, Pb, and Zn using FAAS. Potassium concentrations in above-ground plant and root tissue were analyzed using ion chromatography (Dionex ICS 5000). A total of 4.9 µl of the tissue HNO_3 digestate was mixed with100 µl of methanesulfonic acid and passed through a CS-19 cation exchange column.

Comparison of metal levels (soil or plant tissue) detected in the different treatments was performed using Multivariate Analysis of Variance and Pillai's Trace Test if significant differences were detected ($p < 0.05$). In addition, linear regression analyses were carried out to assess the effects of various factors (e.g., time, rate of application) on rate of metal uptake. SPSS, version 17.0 on a Windows format, was used for statistical analyses.

Field Study

The ability of several plant species, native to the Midwestern United States and showing promise in earlier studies to contribute to metal uptake and/or stabilization (Pichtel and Bradway, 2007) were evaluated in a field study at the former Car Doctor's site.

The field study was conducted using field plots measuring 2 x 3 m each where perennial ryegrass (*Lolium perenne*) was seeded**.** All plots were amended with composted organic material derived from leaves and grass clippings. Plots were sampled at the end of the growing season as described above for the greenhouse study. Both soil material (upper 20 cm) and above-ground plant tissue were extracted and microwave-digested, respectively, as described above.

RESULTS AND DISCUSSION

Greenhouse Study

Soil Characterization

Soil pH was 7.2 and compost pH measured 7.5 (Tables 1). Soil TOC ranged from a low of 0.6% to 13.1% (Table 1). The study site is highly variable in terms of soil chemical and physical properties; it is suspected that the high TOC levels (i.e., those > 2%) are due to anthropogenic effects, i.e., improper release and disposal of fuels and lubricating oils (Cornelissen, et al. 2005). Compost TOC measured 36.8% (Table 1). Soil NO_3^- and NH_4^+ were 1.0 and 1.8 mg/kg, respectively, and levels for the compost were 0.9 and 2.5 mg/kg, respectively. Potassium concentration of the soil and compost were 318.1 mg/kg and 716.5 mg/kg, respectively. Soil texture was a sandy loam (58.4% sand, 32% silt, and 9.7% clay).

Levels of soil extractable metals were within range for most soils (Kabata-Pendias, 2001), with the exception of Cd, which measured 25.1 mg/kg, and Pb, which measured 315.6 mg/kg (Table 1). In non-contaminated soils, Cd concentrations are in the range of 0.1-0.5 mg/kg. Contaminated soils have been found to have Cd concentrations as high as >14,000 mg/kg (Gohre, 2006). At a Superfund site, Pichtel et al. (2000) measured 52 mg/kg soil Cd. In non-contaminated soils, Pb typically does not exceed 70 mg/kg (Pichtel, 2007). Elevated Pb concentrations in soils at this site may be due to atmospheric deposition from nearby industries, or more likely from disposal of Pb-enriched waste.

Concentrations of both Cd and Pb were high in compost (11.3 mg/kg and 172.5 mg/kg, respectively) (Table 1). The reason for elevated Cd and Pb in the compost is not clear.

Table 1. Selected soil and compost chemical and physical properties.

	pH	TOC	NO_3^-	NH_4^+	K
		%	mg/kg		
Soil	7.2 ± 0.8	7.0 ± 6.4*	1 ± 0.4	1.8 ± 0.3	318.1 ± 171.8
Compost	7.5 ± 0	36.8 ± 5.8	0.9 ± 0.1	2.5 ± 0.1	716.5 ± 51.0

* Range of 0.6 % to 13.3%.

Extractable metals

	Cd	Cu	Cr	Ni	Pb	Zn
	mg/kg					
Soil	25.1 ± 23.3	70.6 ± 54.3	8.9 ± 5.7	2.7 ± 2.7	315.6 ± 267.4	92.8 ± 79.0
Compost	11.3 ± 8.4	70.1 ± 7.8	3.8 ± 3.6	3.5 ± 1.5	172.5 ± 92.7	76.5 ± 52.2

Incubations

At 30 d (all plant treatments combined), pH values increased to 7.8 (CB, MF, control), and 7.7 (CB+MF) (Table 2). By 90 d soil pH increased to 8.0 (control). Among individual plant treatments, soil pH of the sunflower treatment was highest at 8.1 (MF, control) and that of the red clover was lowest at 7.7 (MF, CB+MF, control) (Tables 4-5). These values are significantly different ($p < 0.05$). At pH values > approx. 7.5, many soil metals will start to precipitate as oxides, hydroxides, carbonates, etc., thus becoming less available to plants (Bohn et al., 1979).

Table 2. Soil chemical properties, 30 d and 90 d (all plant treatments combined).

Soil Treatment	pH		TOC		NO_3^-	
			%		mg/kg	
	30 d	90 d	30 d	90 d	30 d	90 d
CB[1]	7.8 ± 0.2	7.9 ± 0.2	ND[2]	1.4 ± 0.4	2.9 ± 2.2	1.5 ± 0.9
MF	7.8 ± 0.3	8.0 ± 0.2	ND	1.0 ± 0.3	2.6 ± 2.0	3.3 ± 2.8
CB+MF	7.7 ± 0.1	7.8 ± 0.1	ND	1.3 ± 0.4	2.7 ± 1.8	1.6 ± 1.0
Control	7.8 ± 0.3	8.0 ± 0.2	ND	1.0 ± 0.2	3.1 ± 2.4	4.0 ± 3.5

Soil Treatment	NH_4^+		K	
	---------------------- mg/kg ------------------------			
	30 d	90 d	30 d	90 d
CB	2.9 ± 0.5	1.8 ± 0.3	ND	430.7 ± 188.8
MF	3.7 ± 1.2	1.7 ± 0.3	ND	498.2 ± 182.8
CB+MF	2.6 ± 0.6	1.9 ± 0.4	ND	435.6 ± 145.0
Control	3.3 ± 0.9	1.8 ± 0.2	ND	442.2 ± 164.3

CB[1] = compost; MF = Mycorrhizal fungi.
ND[2] = no data.

Table 3. Soil chemical properties, 30 d and 90 d, ryegrass treatment.

Soil Treatment	pH		TOC		NO_3^-	
			%		------ mg/kg -----	
	30 d	90 d	30 d	90 d	30 d	90 d
CB[1]	7.9 ± 0	8.0 ± 0.1	ND[2]	1.2 ± 0.2	0.9 ± 0.3	0.6 ± 0
MF	7.9 ± 0.1	7.9 ± 0.1	ND	1.0 ± 0.1	1.2 ± 0.2	0.8 ± 0.1
CB+MF	7.7 ± 0.1	7.9 ± 0.1	ND	1.4 ± 0	2.2 ± 1.1	1.0 ± 0.3
Control	7.9 ± 0	8.0 ± 0	ND	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.1

Soil Treatment	NH_4^+		K	
	----------------------- mg/kg -------------------------			
	30 d	90 d	30 d	90 d
CB	3.1 ± 0.2	2.0 ± 0.2	ND	414.5 ± 41.7
MF	2.9 ± 0.4	1.7 ± 0.1	ND	360.2 ± 44.8
CB+MF	2.4 ± 0.4	2.0 ± 0.2	ND	365.0 ± 25.8
Control	3.0 ± 0.1	1.9 ± 0.1	ND	336.9 ± 59.0

CB[1] = compost; MF = mycorrhizal fungi.
ND[2] = no data.

Table 4. Soil chemical properties, 30 d and 90 d, red clover treatment.

Soil Treatment	pH		TOC		NO_3^-	
			%		----- mg/kg ------	
	30 d	90 d	30 d	90 d	30 d	90 d
CB[1]	7.8 ± 0.1	7.9 ± 0.2	ND[2]	1.4 ± 0.3	4.2 ± 0.8	1.7 ± 0.8
MF	7.7 ± 0.2	7.9 ± 0.1	ND	1.1 ± 0.1	2.6 ± 2.0	3.3 ± 2.8
CB+MF	7.7 ± 0.1	7.8 ± 0	ND	1.4 ± 0.3	3.0 ± 0.2	1.6 ± 0.8
Control	7.7 ± 0.3	7.8 ± 0.3	ND	1.0 ± 0.2	3.2 ± 2.2	4.0 ± 3.4

Soil Treatment	NH_4^+		K	
	------------------ mg/kg ---------------------			
	30 d	90 d	30 d	90 d
CB	2.8 ± 0.2	1.6 ± 0.1	ND	384.1 ± 24.2
MF	3.9 ± 0.9	1.7 ± 0	ND	609.9 ± 71.1
CB+MF	2.8 ± 0.4	2.0 ± 0.4	ND	435.6 ± 145.0
Control	2.8 ± 0.5	1.7 ± 0.1	ND	457.3 ± 132.5

CB[1] = compost; MF = mycorrhizal fungi.
ND[2] = no data.

Table 5. Soil chemical properties, 30 d and 90 d, sunflower treatment.

Soil Treatment	pH		TOC		NO_3^-	
			%		------ mg/kg ------	
	30 d	90 d	30 d	90 d	30 d	90 d
CB[1]	7.7 ± 0.1	7.9 ± 0.1	ND[2]	1.5 ± 0.3	0.8 ± 0.2	0.7 ± 0.1
MF	8.0 ± 0.1	8.0 ± 0.2	ND	0.8 ± 0.1	0.9 ± 0.2	0.5 ± 0
CB+MF	7.8 ± 0.1	7.7 ± 0.1	ND	1.0 ± 0.2	1.4 ± 0.4	0.7 ± 0.1
Control	8.0 ± 0.1	8.1 ± 0	ND	0.9 ± 0	1.0 ± 0.4	0.6 ± 0.1

Soil Treatment	NH_4^+		K	
	------------------ mg/kg -------------------			
	30 d	90 d	30 d	90 d
CB	2.8 ± 0.4	1.7 ± 0.2	ND	270.3 ± 28.4
MF	2.9 ± 0.4	1.5 ± 0.1	ND	576.5 ± 15.9
CB+MF	2.3 ± 0	1.8 ± 0.3	ND	483.8 ± 39.3
Control	3.8 ± 0.4	1.6 ± 0.1	ND	535.9 ± 70.6

CB[1] = compost; MF = mycorrhizal fungi.
ND[2] = no data.

Table 6. Soil chemical properties, 30 d and 90 d, no plant growth (control).

Soil Treatment	pH		TOC		NO_3^-	
			%		----- mg/kg -----	
	30 d	90 d	30 d	90 d	30 d	90 d
CB[1]	7.7 ± 0.1	8.0 ± 0	ND[2]	1.5 ± 0.3	2.0 ± 1.1	1.4 ± 0.2
MF	7.7 ± 0.1	7.9 ± 0	ND	1.2 ± 0.1	1.7 ± 0.6	1.9 ± 0.9
CB+MF	7.7 ± 0.1	7.8 ± 0.1	ND	1.6 ± 0	3.1 ± 1.4	1.0 ± 0.4
Control	7.8 ± 0	8.0 ± 0	ND	1.1 ± 0.1	1.5 ± 0.4	1.1 ± 0.4

Soil Treatment	NH_4^+		K	
	------------------ mg/kg -----------------			
	30 d	90 d	30 d	90 d
CB	3.1 ± 0.3	1.6 ± 0.1	ND	496.5 ± 122.9
MF	3.1 ± 0.2	1.8 ± 0.2	ND	355.4 ± 33.7
CB+MF	2.8 ± 0.2	1.8 ± 0	ND	499.5 ± 40.8
Control	3.3 ± 0.6	1.7 ± 0.1	ND	402.3 ± 103.6

CB[1] = compost; MF = mycorrhizal fungi.
ND[2] = no data.

By 90 d (all plant treatments combined), TOC was lowest in the MF treatment (1.0%) and highest in the CB treatment (1.4%). Control soil (CB+MF) had the highest TOC at 1.6%, and the control soil (CB) contained 1.5 % TOC (Table 2). The lowest soil TOC value occurred in the sunflower (MF) treatment at 0.8% (Table 5). Bowman et al. (1999) found that continuous cropping of a cereal-based soil increased TOC by 20% from 0-5 cm soil depth and increased soluble organic carbon by about

33%. Italian ryegrass (*Lolium multiflorum*) was used in a re-colonization study by Ye et al. (2000) due to its ability to quickly increase soil TOC levels.

At 30 d (all plant growth combined), soil NO_3^- was highest in the control (3.1 mg/kg) and lowest in the MF treatment (2.6 mg/kg) (Table 2).

Nitrate concentrations in the sunflower treatment (CB and MF) were lowest at 0.8 mg/kg and 0.86 mg/kg, respectively (Table 5). Highest NO_3^- concentrations were measured in the red clover treatment CB at 4.2 mg/kg (Table 4). Clover (*Trifolium repens*) species have been used successfully in several plant re-colonization studies for its ability to solubilize soil nitrogen (Ye et al., 2000).

At 90 d (all plant treatments combined), lowest soil NO_3^- concentrations were measured in the C treatment (1.5 mg/kg) and the highest in the control (4.0 mg/kg) (Table 2). Soil in the sunflower treatment (MF) contained lowest NO_3^- concentrations (0.5 mg/kg), a 36% decline compared with 30 d values (Table 5). Highest soil NO_3^- concentrations were measured in the red clover CB treatment at 4.0 mg/kg, which declined by 4.7% from the 30 d concentration (4.2 mg/kg) (Table 4). The NO_3^- concentrations for each species between 30 d and 90 d are not signifigant ($p > 0.05$). Wallgren and Linden (1994) found that red clover-rye mixture increased soil nitrate and ammonium levels, thus increasing subsequent barley crop grain yields by 10-16%. In a study by Pan et al. (2013) it was found that an increase of sand content decreased total soil NO_3^- levels. The study soil contains 58.4% sand (Table 1). Several studies have determined substantial NO_3^- leaching from fertilized turfgrass (Frank et al., 2008; Morton et al., 1988; Owen and Barraclough, 1983; Brown et al., 1982; Rieke and Ellis, 1974). Single dose, high rate, water-soluble N applications to mature turf grass stands tend to result in excess N losses as NO_3^- (Frank et al., 2008).

At 30 d soil ammonium concentrations (all plant treatments combined) were highest in the MF soil treatment at 3.7 mg/kg; lowest soil NH_4^+ was in CB+MF (2.6 mg/kg) (Table 2). Soil in the red clover treatment (MF) had highest NH_4^+ concentrations (3.9 mg/kg) and ryegrass (CB+MF) the lowest at 2.4 mg/kg (Tables 3-4). By 90 d soil NH_4^+ concentrations were highest in the CB+MF (1.9

mg/kg and MF was lowest at 1.7 mg/kg); although all soil treatment ranges had decreased NH_4^+ concentrations overall) (Table 2).

From 90 d red clover (CB+MF) had the greatest NH_4^+ at 2.0 mg/kg while sunflower (MF) had the lowest at 1.5 mg/kg (Tables 4-5). The NH_4^+ concentrations for each species between 30 and 90 d are significant ($p < 0.05$). Brown et al. (1982) noted negligible NH_4^+ losses from golf greens treated with several N fertilizer types. Mengel and Scherer (1981) measured a decline in soil NH_4^+ during the growing season followed by a subsequent rebound due to release from exchangeable form.

Soil Metals

Cadmium

At 90 d soil Cd concentrations were highest in the CB+MF treatment (all plant treatments combined), at 94.5 mg/kg (Table 7). Soil Cd ranged between 14.1-14.8 mg/kg in the CB, MF, and control treatments. Root exudates of *Nicotiana* spp. were found to enhance the solubility of Cd in soil (Mench and Martin, 1991).

Regardless of soil treatment, soil Cd concentrations remained < 15.5 mg/kg in the red clover and sunflower treatments (Tables 9-10). Highest soil Cd was measured in the ryegrass treatment, with concentrations ranging between 15.5 mg/kg (MF) and 73.0 mg/kg (CB+MF) (Table 8).

Table 7. Soil extractable metals, 90 d (all plant treatments combined).

	Cd	Cu	Cr	Ni	Pb	Zn
	------ mg/kg ------					
Treatment						
CB	14.3 ± 8.5	217.6 ± 76.0	24.8 ± 5.6	23.3 ± 3.7	603.7 ± 283.9	622.7 ± 235.0
MF	14.8 ± 7.9	230.0 ± 74.1	22.4 ± 8.3	20.1 ± 7.9	696.9 ± 270.3	525.1 ± 184.7
CB+MF	94.5 ± 87.5	244.3 ± 96.2	21.6 ± 15.2	19.5 ± 11.3	616.0 ± 203.5	662.1 ± 221.1
Control	14.1 ± 5.38	200.3 ± 61.7	24.4 ± 10.0	22.0 ± 9.0	605.6 ± 241.2	578.3 ± 164.5

Table 8. Soil extractable metals, 90 d, ryegrass treatment.

	Cd	Cu	Cr	Ni	Pb	Zn
	------ mg/kg ------					
Treatment						
CB	18.3 ± 4.4	259.5 ± 34.2	27.9 ± 1.9	20.7 ± 1.0	601.3 ± 60.1	627.5 ± 32.9
MF	15.5 ± 7.3	147.2 ± 21.8	30.0 ± 0.7	20.5 ± 1.1	480.9 ± 54.3	391.5 ± 51.0
CB+MF	73.0 ± 66.0	184.0 ± 8.0	29.0 ± 1.2	21.0 ± 2.2	482.5 ± 32.4	714.2 ± 169.0
Control	10.8 ± 2.0	252.3 ± 6.2	32.2 ± 0.3	19.2 ± 1.5	447.9 ± 83.6	426.4 ± 12.6

Table 9. Soil extractable metals, 90 d, red clover treatment.

Treatment	Cd	Cu	Cr	Ni	Pb	Zn
	-- mg/kg --					
CB	14.5 ± 6.2	217.0 ± 35.0	21.1 ± 1.9	25.5 ± 1.5	557.6 ± 27.8	659.1 ± 45.4
MF	13.3 ± 0.7	200.3 ± 8.0	24.9 ± 1.7	23.4 ± 4.6	867.7 ± 99.5	669.0 ± 40.8
CB+MF	13.4 ± 1.0	157.1 ± 9.0	10.6 ± 4.2	15.7 ± 7.4	627.8 ± 211.5	535.2 ± 31.9
Control	15.5 ± 4.0	213.1 ± 48.9	30.4 ± 3.9	26.0 ± 4.9	622.8 ± 23.9	580.5 ± 37.0

Table 10. Soil extractable metals, 90 d, sunflower treatment.

Treatment	Cd	Cu	Cr	Ni	Pb	Zn
	-- mg/kg --					
CB	7.0 ± 1.2	190.0 ± 48.5	28.6 ± 1.0	21.2 ± 1.6	360.9 ± 41.1	470.1 ± 82.4
MF	12.2 ± 5.3	172.5 ± 16.5	16.2 ± 2.1	17.5 ± 5.3	802.5 ± 23.8	573.3 ± 37.7
CB+MF	15.1 ± 2.5	229.1 ± 55.8	31.9 ± 5.0	24.3 ± 6.5	665.6 ± 68.0	558.3 ± 117.2
Control	14.3 ± 3.8	173.2 ± 34.6	17.5 ± 3.1	17.3 ± 4.3	753.6 ± 93.2	528.4 ± 27.9

Table 11. Soil extractable metals, 90 d, no plant growth (control).

	Cd	Cu	Cr	Ni	Pb	Zn
	-- mg/kg					--
Treatment						
CB	17.0 ± 1.5	224.3 ± 28.5	25.6 ± 4.8	21.8 ± 2.0	718.5 ± 169.2	781.3 ± 76.3
MF	10.1 ± 1.3	163.2 ± 3.5	29.6 ± 0.5	20.0 ± 1.8	464.6 ± 34.4	454.6 ± 25.3
CB+MF	96.1 ± 86.0	295.5 ± 45.0	11.3 ± 3.5	24.2 ± 3.5	705.3 ± 48.2	709.1 ± 29.8
Control	12.9 ± 2.4	199.0 ± 51.0	31.2 ± 1.4	23.5 ± 6.3	552.8 ± 137.8	678.4 ± 50.5

Copper

At 90 d soil Cu concentrations were highest for the CB+MF treatment (all plant treatments combined) at 244.3 mg/kg and lowest in the control at 200.3 mg/kg (Table 7). Excess Cu in soil has been hypothesized as limiting the phytoextraction of multiple metals by plants (Gunawardana et al. 2011, Padmavathiamma and Li, 2007).

In individual species treatments, the control soil (CB+MF) contained the highest Cu concentration at 295.5 mg/kg; lowest Cu was measured in ryegrass (MF) at 147.2 mg/kg (Tables 8,11). Copper is highly susceptible to immobilization in the presence of organic matter and/or at neutral-high pH (Padmavathiamma and Li, 2007). The study soil was high in organic matter and was slightly alkaline in pH (Table 1).

Chromium

At 90 d soil Cr concentrations were similar (all plant treatments combined), with highest concentrations at 24.8 mg/kg (CB) and lowest at 21.6 mg/kg (MF) (Table 7).

Soil in the ryegrass (control) treatment contained highest Cr concentrations (32.2 mg/kg) while lowest Cr concentrations were detected in the red clover treatment (CB+MF) at 10.6 mg/kg (Tables 8-

9). These differences are not significantly different ($p > 0.05$). Sunflower (CB+MF) contained 31.9 mg/kg soil Cr (Table 10). The red clover treatment showed the most variability among plant treatments in soil Cr concentrations (Table 9).

Nickel

At 90 d soil Ni concentrations (all plant treatments combined) were highest in the CB treatment (23.3 mg/kg) and control (20.1 mg/kg) (Table 7). Uncontaminated soils generally contain a wide range of Ni concentrations, depending on parent material, averaging 100 mg/kg (Hutchinson, 1981; Padmavathiamma and Li, 2007).

Red clover (control) soil contained highest Ni at 26.0 mg/kg, while the red clover (CB+MF) treatment contained lowest soil Ni at 15.7 mg/kg (Table 9). Sanders et al. (2006) found that increasing soil pH from 4.5 to 7.5 resulted in decreased concentrations of extractable Ni. At 90 d (all soil treatments combined) soil pH ranged from 7.7-8.0 (Table 2).

Lead

At 90 d soil Pb (MF, all plant treatments combined) concentrations were highest at 696.9 mg/kg and lowest in the CB soil treatment at 603.7 mg/kg (Table 7). The red clover (MF) treatment contained the greatest Pb concentration at 867.7 mg/kg. This is despite the fact that soil pH in this treatment was 7.9 (Table 4). Soil in the sunflower (MF) treatment contained 802.5 mg/kg while sunflower (CB) had the lowest Pb concentration, i.e., 360.9 mg/kg (Table 10).

Zinc

At 90 d the CB+MF treatment (all plant treatments combined) contained the greatest soil Zn concentrations (662.1 mg/kg) while MF contained the lowest concentrations (525.1 mg/kg) (Table 7). Even though soil Zn concentrations of the present study are the result after plant treatment, this soil would still be considered highly contaminated. Concentrations of 17-125 mg/kg occur naturally in soils (Pichtel, 2007).

The control (CB) treatment soil contained the highest Zn concentration (781.3 mg/kg) while ryegrass (MF) soil contained the lowest soil Zn (391.5 mg/kg) (Tables 8,11). Ryegrass (CB+MF) soil also contained high concentrations of Zn (714.2 mg/kg).

Plant tissue metals

Cadmium

At 30 d the red clover treatment (all soil treatments combined) contained highest tissue Cd at 27.7 mg/kg; ryegrass contained the lowest concentration at 15.9 mg/kg (Table 12). By 90 d sunflower contained the highest tissue Cd at 45.2 mg/kg. This tissue Cd concentration was higher than that found using maize and Indian mustard (4 and 15 mg/kg, respectively) found by Bricker et al. (2001). This value of tissue Cd is substantial; however, the plant does not quality as a hyperaccumulator.

Brown et al. (1995) found that *Thlaspi caerulescens* concentrated about 1140 mg/kg Cd in its biomass. Jarvis et al. (1976) found that mature ryegrass translocated minimal Cd to roots in the first three days of exposure to Cd but did not translocate Cd to the shoots for the following 21 days. They conclude that even though several species may take up increased Cd in roots, they may not possess the mechanisms to move Cd to shoots. Red clover translocated Cd from root to shoot, as shown by the high Cd concentration in above-ground biomass from 30 d to 90 d (Figure 3) (Jarvis et al., 1976). Williams and David (1977) found that Cd added to soil (between 5 to 100 mg/kg) matched the increasing amounts of Cd taken up by red clover. When Cd was added only to the top 2 cm of soil, root biomass below 2 cm was unaffected but Cd immobilization in the affected root area may have reduced Cd translocation to shoots (Williams and David, 1977).

Table 12. Plant tissue metals, 30d and 90 d (all soil treatments combined).

	Cd		Cu		Cr	
	-- mg/kg --					
Plant type	30 d	90 d	30 d	90 d	30 d	90 d
Ryegrass	15.9 ± 7.4	39.2 ± 6.5	953.9 ± 779.9	221.3 ± 114.7	10.3 ± 6.9	41.6 ± 37.8
Red clover	27.7 ± 20.3	43.1 ± 8.5	1063.3 ± 887.1	575.4 ± 435.5	37.6 ± 28.5	69.4 ± 61.0
Sunflower	21.0 ± 10.5	45.2 ± 9.1	738.3 ± 530.9	267.2 ± 122.2	34.2 ± 23.0	25.5 ± 21.7

	Ni		Pb		Zn	
	-- mg/kg --					
Plant type	30 d	90 d	30 d	90 d	30 d	90 d
Ryegrass	72.3 ± 31.0	53.3 ± 9.2	34.1 ± 33.0	95.4 ± 86.5	1242.5 ± 507.5	567.3 ± 183.8
Red clover	111.6 ± 69.1	71.8 ± 31.7	198.2 ± 69.3	293.2 ± 269.6	903.5 ± 450.5	675.3 ± 320.9
Sunflower	90.1 ± 42.3	52.1 ± 12.4	23.3 ± 17.1	131.6 ± 98.4	1016.0 ± 526.2	582.8 ± 137.5

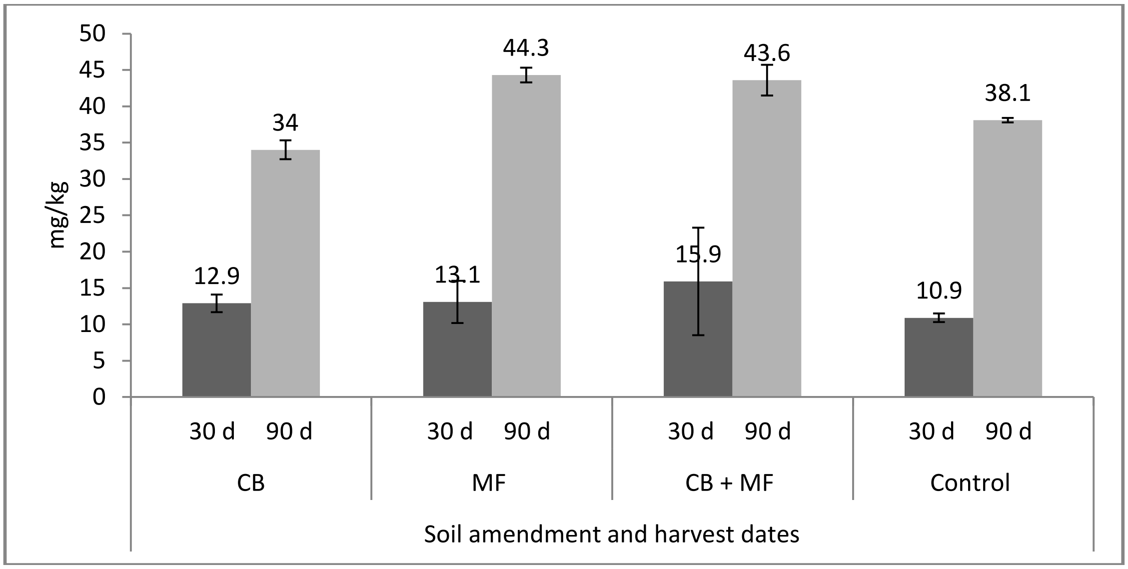

Figure 2. Cadmium concentration in above-ground biomass, ryegrass treatment, 30 d and 90 d.

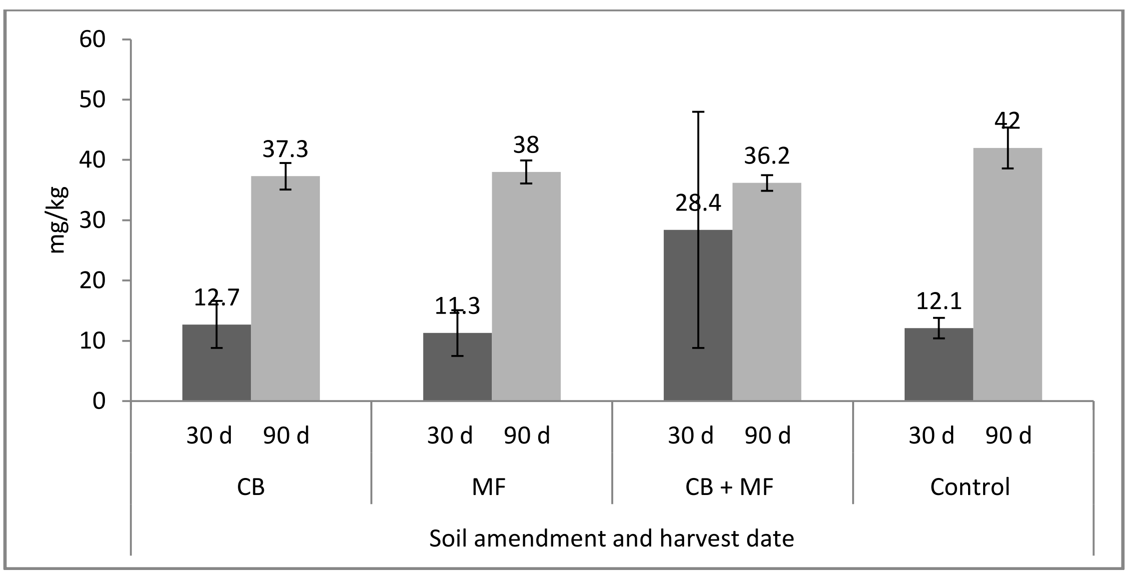

Figure 3. Cadmium concentration in above-ground biomass, red clover treatment, 30 d and 90 d.

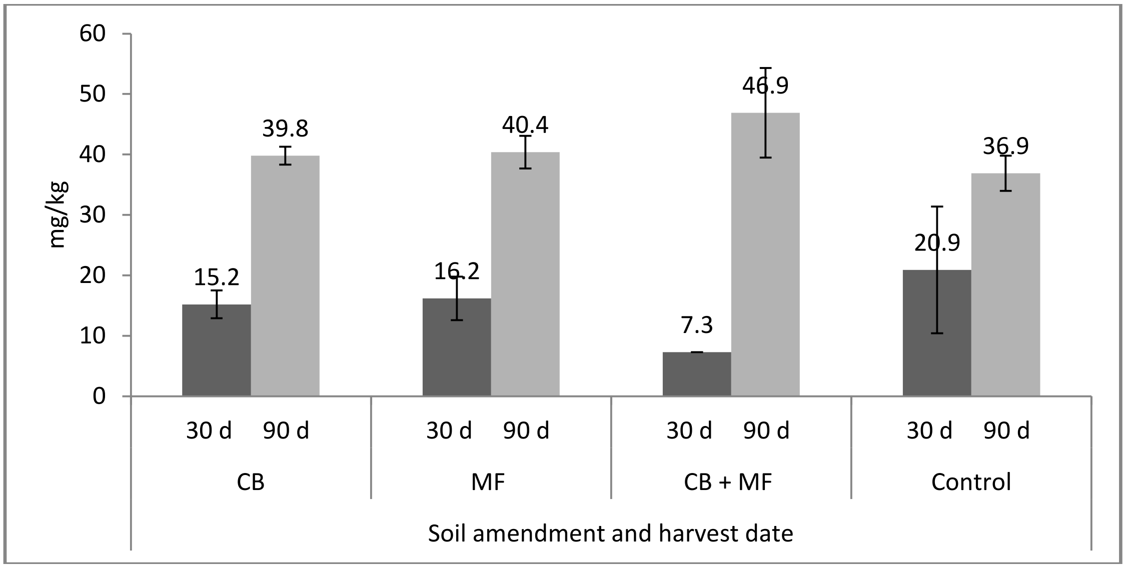

Figure 4. Cadmium concentration in above-ground biomass, sunflower treatment, 30 d and 90 d.

At 30 d (all soil and plant treatments), Cd values ranged from 7.3 mg/kg (sunflower, CB+MF) to 28.4 mg/kg (red clover, CB+MF) (Figs. 3-4). Agricultural crops vary across species in regards to quantity of Cd taken up (Webber, 1981). Sunflower (CB+MF) from 90 d concentrated the most Cd at 46.9 mg/kg (Fig. 4). This value of tissue Cd is not signifigant ($p > 0.05$). Soil Cd can become mobilized with additions of carbonaceous materials (Padmavathiamma and Li, 2007; Webber, 1981). Biosolid compost application has been documented as decreasing Cd uptake by plants due to Cd becoming complexed with organic matter, while leaf compost application can result in weak organic complexes or bioavailable, free ionic form Cd (Bolan et al., 2002; Martinez and McBride, 1999). Soil TOC at the site ranged from 0.6% to 13.3% (Table 1).

Several plant and soil treatment combinations resulted in root Cd concentrations below detectable limits, including ryegrass (CB), red clover (CB, MF, CB+MF), and sunflower (CB, MF, control) (Tables 14-16). High concentrations of soil Cd have been shown to inhibit MF germination and hyphal development prior to plant establishment (Gohre, 2006). At 90 d ryegrass roots (all soil treatments combined) contained highest Cd (11.7 mg/kg) while sunflower roots contained the lowest quantities (0.4 mg/kg Cd) (Table 13). The high Cd concentration in ryegrass soil (CB+MF) (73.0 mg/kg) along with its high Cd root concentration may indicate that ryegrass may impart a Cd stabilizing effect rather than an extraction effect (Gunawardana et al., 2011).

Table 13. Extractable metals from plant roots, 90 d (all soil treatments combined).

	Cd	Cu	Cr	Ni	Pb	Zn
	mg/kg					
Plant type						
Ryegrass	11.7 ± 5.0	419.9 ± 185.2	196.6 ± 133.3	85.3 ± 27.0	627.1 ± 460.3	929.1 ± 261.2
Red clover	3.6 ± 0.7	278.7 ± 177.1	379.2 ± 225.0	113.2 ± 74.0	644.4 ± 362.6	674.6 ± 355.1
Sunflower	0.4 ± 1.3	816.0 ± 581.3	342.1 ± 205.8	72.4 ± 31.2	656.6 ± 294.0	332.6 ± 153.8

Table 14. Extractable metals from ryegrass roots, 90 d.

	Cd	Cu	Cr	Ni	Pb	Zn
	mg/kg					
Treatment						
CB	BDL*	344.7 ± 6.7	146.6 ± 83.3	94.3 ± 17.8	300.1 ± 133.3	714.6 ± 46.7
MF	13.6 ± 3.1	568.3 ± 29.0	206.3 ± 63.2	83.1 ± 12.4	403.0 ± 229.7	1100.7 ± 89.7
CB+MF	9.7 ± 2.9	307.9 ± 36.4	202.7 ± 57.5	85.3 ± 27.0	281.8 ± 100.9	942.0 ± 101.4
Control	8.9 ± 1.3	439.9 ± 111.1	300.0 ± 30.0	96.5 ± 5.1	977.4 ± 110.0	1056.5 ± 25.8

BDL* = Below detectable limit.

Table 15. Extractable metals from red clover roots, 90 d.

Treatment	Cd	Cu	Cr	Ni	Pb	Zn
	mg/kg					
CB	BDL*	157.1 ± 55.5	379.2 ± 225.0	58.0 ± 20.8	174.9 ± 64.0	131.2 ± 11.6
MF	BDL	246.6 ± 67.4	252.9 ± 95.2	123.0 ± 64.2	306.5 ± 94.0	374.2 ± 69.5
CB+MF	BDL	167.3 ± 26.8	181.8 ± 9.8	42.5 ± 3.3	445.6 ± 24.0	165.2 ± 38.4
Control	14.4 ± 2.9	413.5 ± 42.3	238.9 ± 19.0	88.0 ± 16.6	360.7 ± 259.8	1128.0 ± 101.7

BDL* = Below detectable limit.

Table 16. Extractable metals from sunflower roots, 90 d.

Treatment	Cd	Cu	Cr	Ni	Pb	Zn
	mg/kg					
CB	BDL*	363.0 ± 94.4	247.5 ± 52.6	53.3 ± 12.0	545.3 ± 101.7	189.1 ± 14.6
MF	BDL	362.8 ± 128.2	306.4 ± 79.4	79.5 ± 22.9	699.3 ± 309.7	358.0 ± 82.5
CB+MF	1.7 ± 0	921.0 ± 103.2	138.9 ± 2.6	42.8 ± 0.9	147.4 ± 132.9	339.7 ± 54.4
Control	BDL	442.2 ± 80.8	449.6 ± 98.3	114.1 ± 16.8	796.8 ± 301.9	460.8 ± 29.9

BDL* = Below detectable limit.

Copper

At 30 d red clover (all soil treatments combined) contained the greatest concentration of Cu (1063.3 mg/kg) while sunflower contained the lowest concentration (738.3 mg/kg) (Table 12). These concentrations are not signifigantly ($p > 0.05$) different. CB+MF had a significant effect ($p < 0.05$) on Cu uptake in ryegrass and sunflower compared to CB and MF alone (Figs. 5,7). Clemente et al. (2003; 2006) found that several organic amendments including compost increased Cu uptake by *B.*

juncea. Increasing soil pH does not significantly decrease Cu bioavailability (Padmavathiamma and Li, 2007). By 90 d red clover (CB+MF) took up the most Cu (438.0 mg/kg) while ryegrass (CB+MF) concentrated the lowest quantity (126.4 mg/kg) (Figs. 5-6).

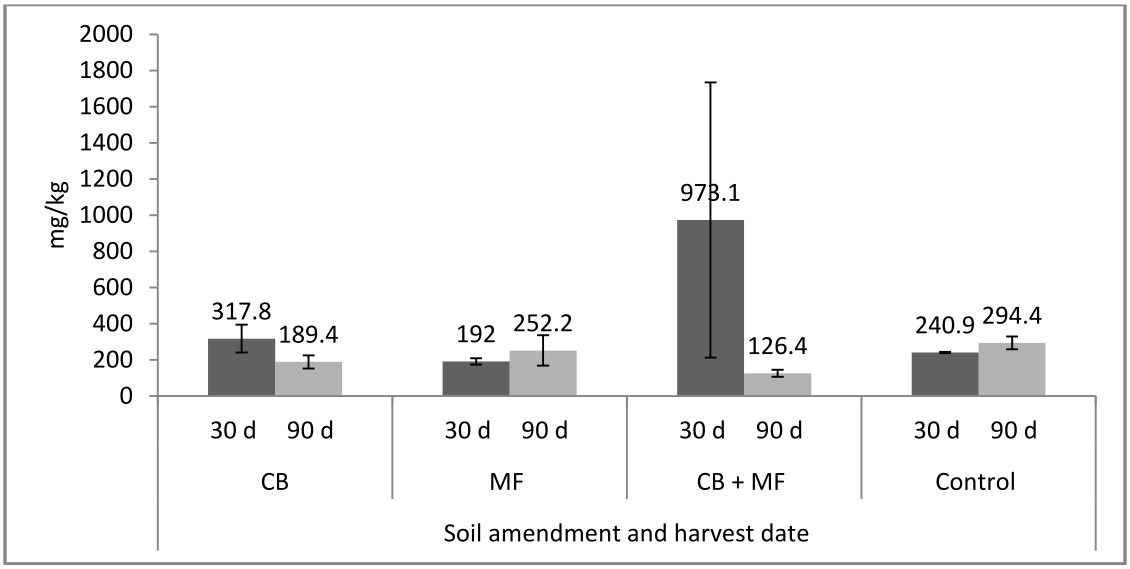

Figure 5. Copper concentration in above-ground biomass, ryegrass treatment, 30 d and 90 d.

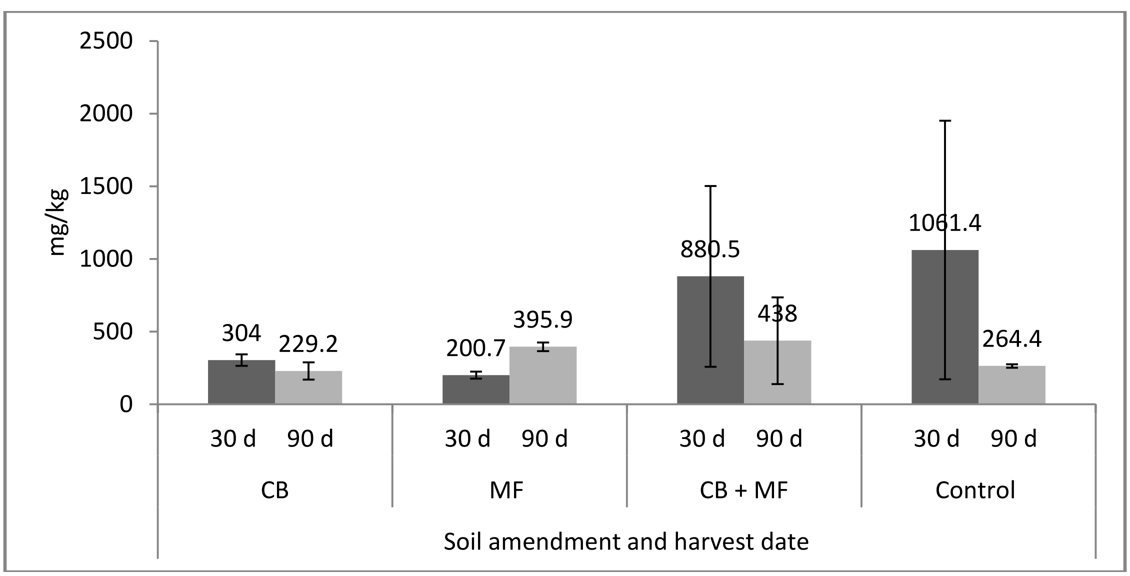

Figure 6. Copper concentration in above-ground biomass, red clover treatment, 30 d and 90 d.

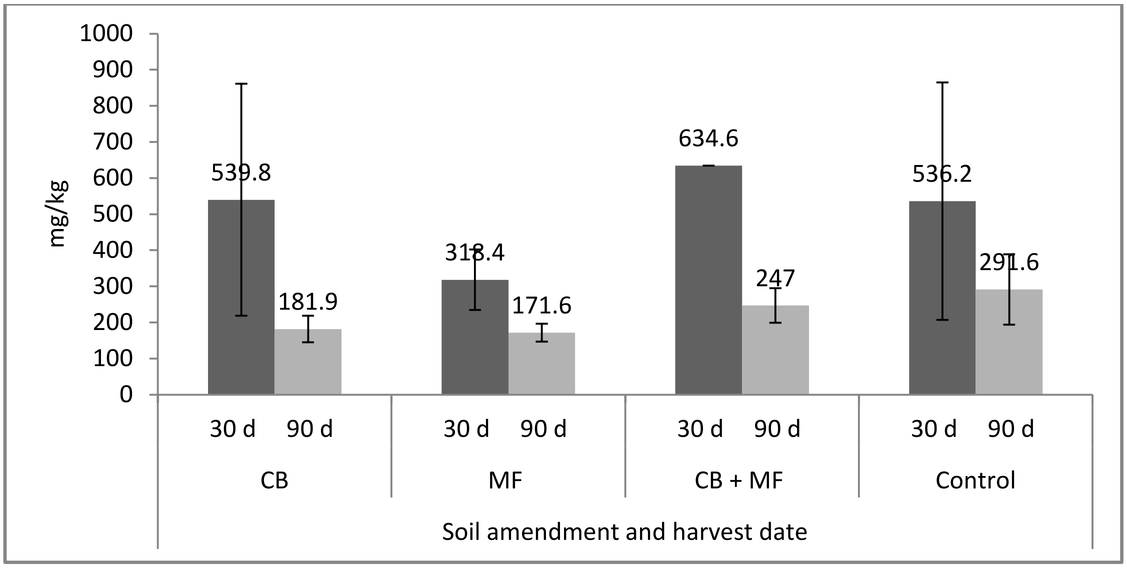

Figure 7. Copper concentration in above-ground biomass, sunflower treatment, 30 d and 90 d.

At 90 d sunflower (all soil treatments combined) concentrated the most Cu in its roots (816.0 mg/kg) and red clover concentrated the least (278.7 mg/kg) (Table 13). Ryegrass (CB+MF) and red clover roots (CB+MF) contained significantly more ($p < 0.05$) Cu than did roots in the MF or CB treatments (Tables 14-15). Freedman (1980) found that soil Cu concentrations >1000 mg/kg imparted a negative effect on fungal respiration (Hutchinson, 1981). Sunflower (CB+MF) roots concentrated the most Cu at 921 mg/kg while red clover (CB) concentrated the least at 157.1 mg/kg (Table 15-16). These values are significantly different ($p < 0.05$).

Chromium

At 30 d ryegrass (all soil treatments combined) took up the lowest amount of Cr (10.3 mg/kg) (Table 12). In a greenhouse study, Pichtel and Salt (1998) found that ryegrass contained up to 359 mg/kg Cr. Different varieties of ryegrass may take up different quantities of soil metals; furthermore, total soil Cr in the Pichtel and Salt (1998) paper was substantially greater (3540 mg/kg, compared with 8.9

mg/kg in the current study (Table 1). Red clover concentrated the most Cr at both 30 d (37.6 mg/kg) and 90 d (69.4 mg/kg) (Table 12).

At 30 d, red clover (CB) concentrated the most Cr at 42.1 mg/kg while sunflower (CB+MF) concentrated the least at 5.8 mg/kg (Figs. 9-10). Red clover (MF) experienced an 85% increase in Cr uptake from 30 d (15.1 mg/kg) to 90 d (100.5 mg/kg) (Fig. 9). Ryegrass (CB) experienced a 79% increase in uptake from 30 d (15.6 mg/kg) to 90 d (75.0 mg/kg) (Fig. 8). Both uptake increases were significant ($p < 0.05$).

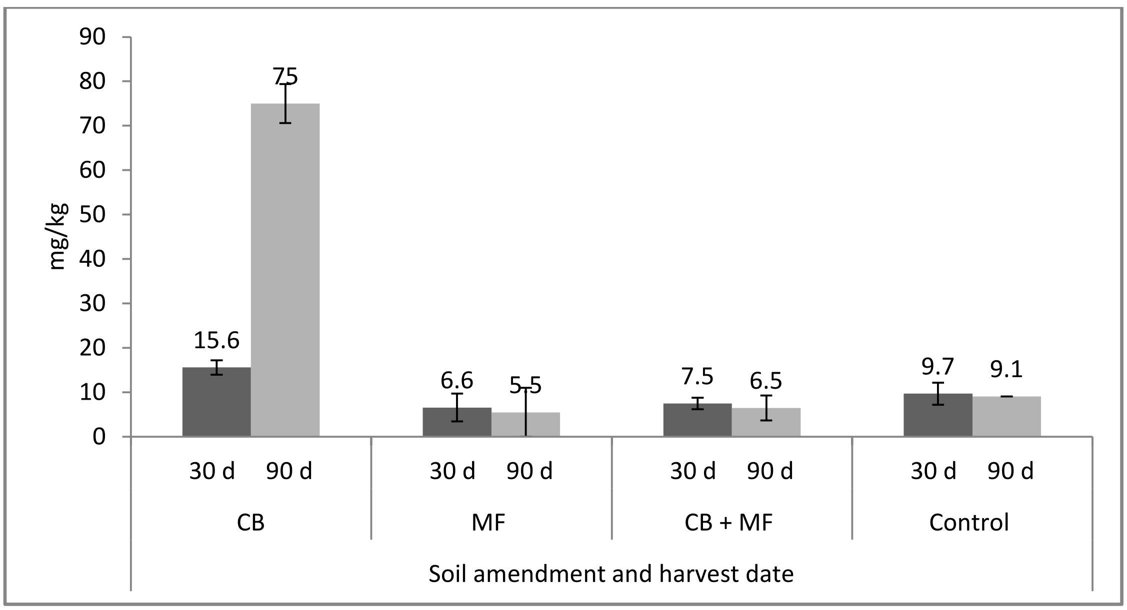

Figure 8. Chromium concentration in above-ground biomass, ryegrass treatment, 30 d and 90 d.

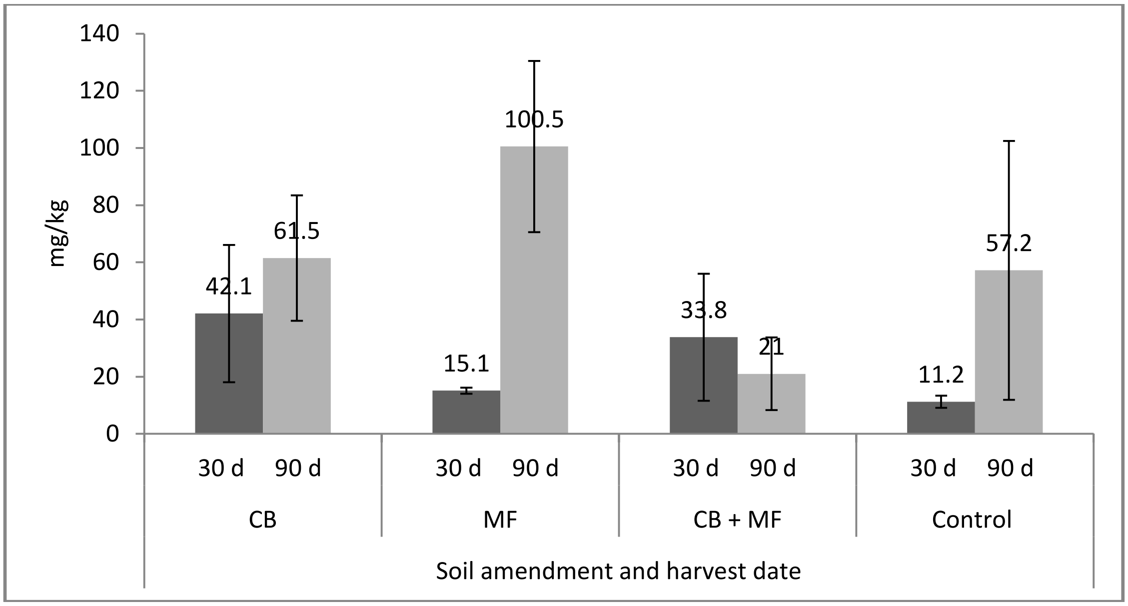

Figure 9. Chromium concentration in above-ground biomass, red clover treatment, 30 d and 90 d.

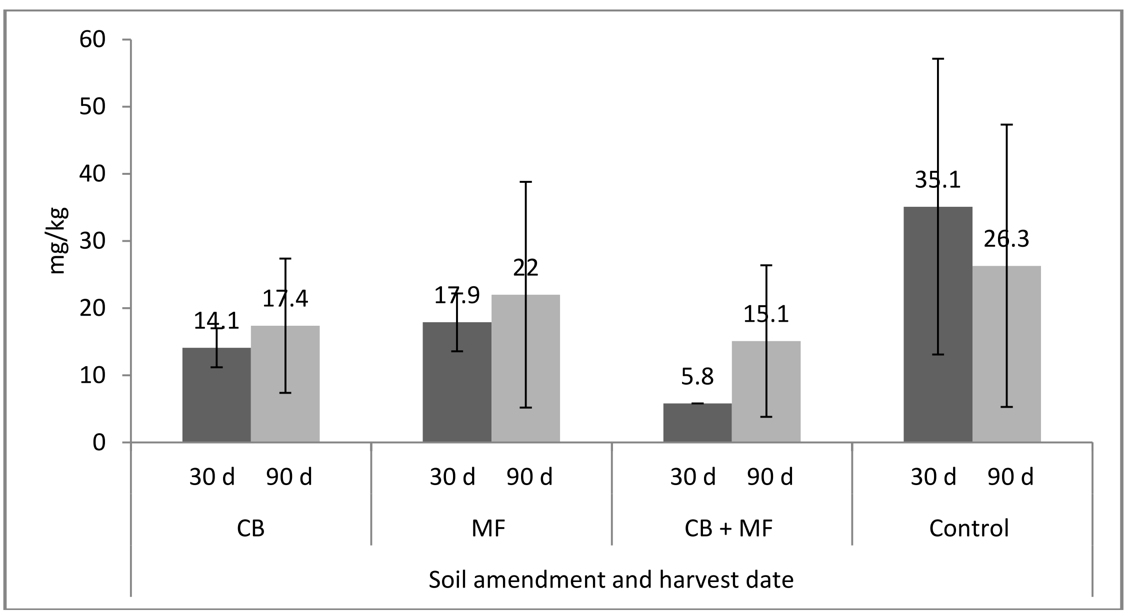

Figure 10. Chromium concentration in above-ground biomass, sunflower, 30 d and 90 d.

At 90 d sunflower roots (all soil treatments combined) contained the greatest quantity of Cr (285.6 mg/kg) and ryegrass contained the least (213.9 mg/kg) (Table 13).

Red clover roots (CB) contained the most Cr at 379.2 mg/kg (Table 15). Root Cr concentrations from all plant-soil treatment combinations were higher than were Cr concentrations in above-ground biomass (Table 15). This phenomenon is not considered unusual. Zhu et al. (1999) found that water hyacinth (*Eichhornia crassipes*) concentrated Cr at concentrations of 119 mg/kg in shoots while containing 3,951 mg/kg in roots (Padmavathiamma and Li, 2007).

Nickel

At 30 d red clover (all soil treatments combined) contained the greatest quantity of Ni at 111.6 mg/kg; ryegrass contained the lowest quantity at 72.3 mg/kg (Table 12). Tissue Ni concentrations of 50 mg/kg can be phytotoxic in certain species. This degree of Ni uptake is possible even in soil Ni concentrations <10 mg/kg for several species (Hutchinson, 1981).

Red clover (CB+MF) contained the greatest quantity of Ni at 112.7 mg/kg, and sunflower (control) contained 95.5 mg/kg (Fig. 12). Bertoloni columbine (*Aquilegia bertolonii*) and Australian spinach (*Chenopodiastrum Murale*) concentrated up to 10% Ni (dry weight basis) in its biomass, while *Cotoneaster acuminatus* concentrated up to 25% of its biomass as Ni (Freedman and Hutchinson, 1981).

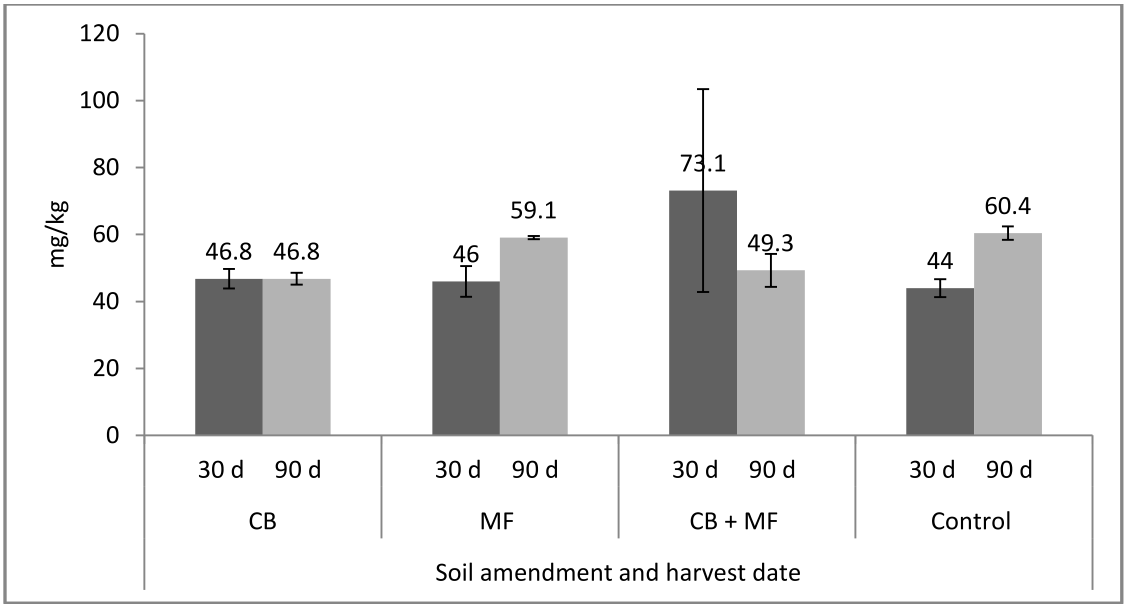

Figure 11. Nickel concentration in above-ground biomass, ryegrass treatment, 30 d and 90 d.

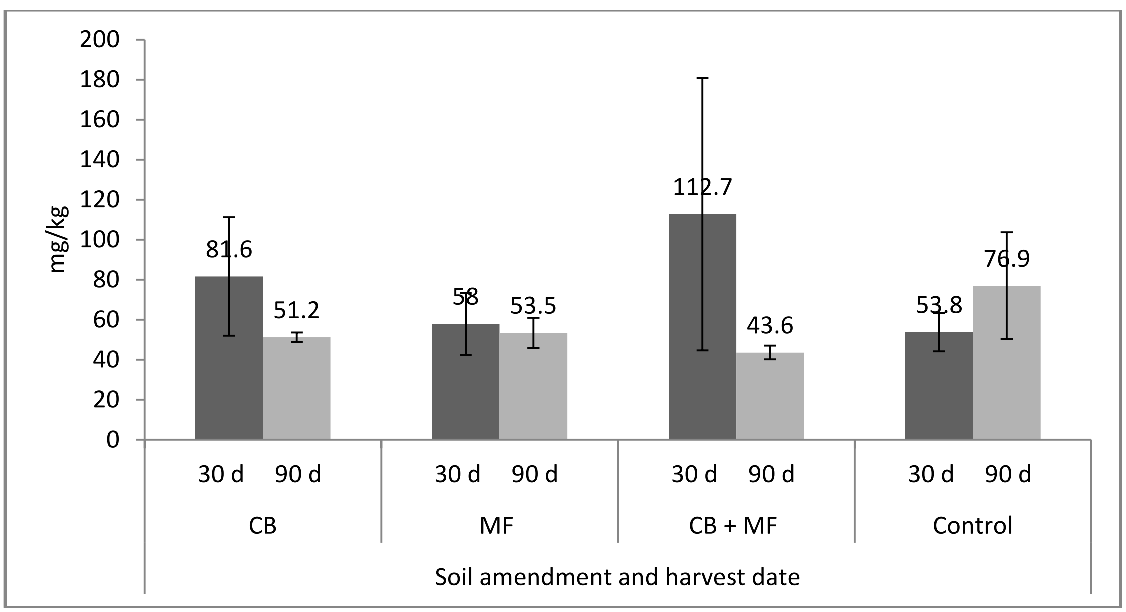

Figure 12. Nickel concentration in above-ground biomass, red clover treatment, 30 d and 90 d.

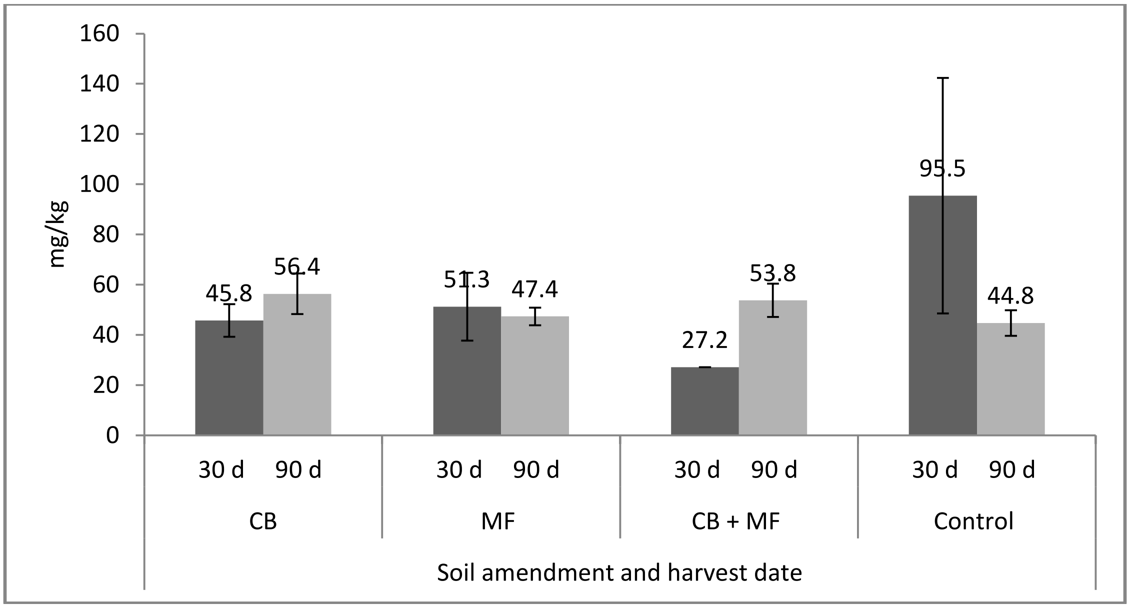

Figure 13. Nickel concentration in above-ground biomass, sunflower treatment, 30 d and 90 d.

Nickel uptake by red clover (CB+MF) was reduced from 112.7 mg/kg at 30 d to 43.6 mg/kg at 90 d, a reduction of 61% (Fig. 12). This reduction, which is not significant ($p > 0.05$), may possibly be a result of MF or plant mechanisms that halted Ni uptake before it could become phytotoxic (Gohre and Paszkowski, 2006).

At 90 d red clover roots (all soil treatments combined) contained the most Ni at 113.2 mg/kg while ryegrass and sunflower roots contained 85.3 and 72.4 mg/kg, respectively (Table 13).

Red clover roots (MF) contained the greatest quantity of Ni (123.0 mg/kg) and sunflower roots (control) concentrated 114.1 mg/kg (Tables 15-16). Mitchell (1945) reported 1-9 mg/kg Ni in red clover (*Trifolium pretense*) above-ground tissue, and up to 46 mg/kg in timothy (*Phleum pretense*) above-ground tissue (Hutchinson, 1981). Red clover roots (CB+MF) contained lowest Ni concentrations (42.5 mg/kg) (Table 15).

Lead

At 30 d red clover (all soil treatments combined) concentrated the greatest quantity of Pb at 198.2 mg/kg while ryegrass and sunflower concentrated significantly less ($p < 0.05$) Pb at 34.1 mg/kg and

23.3 mg/kg, respectively (Table 12). The quantity of tissue Pb in red clover is promising for phytoextraction purposes. This species, however, does not qualify as a hyperaccumulator of soil Pb.

Several plant and soil treatment combinations at 30 d resulted in non-detectable uptake of Pb, including ryegrass (CB+MF, control), red clover (MF), and sunflower (CB+MF) (Figs. 14-16). Many plant species are known to restrict Pb uptake and/or translocation to above-ground biomass (Memon and Shroder, 2009). Furthermore, an antagonistic effect of multiple metal uptake, especially between Cd and Pb, may have occurred that prevented plants from taking up Pb (Gunawardana et al., 2011). Some of the above treatments may, therefore, be better suited to stabilization, rather than extraction of soil metal.

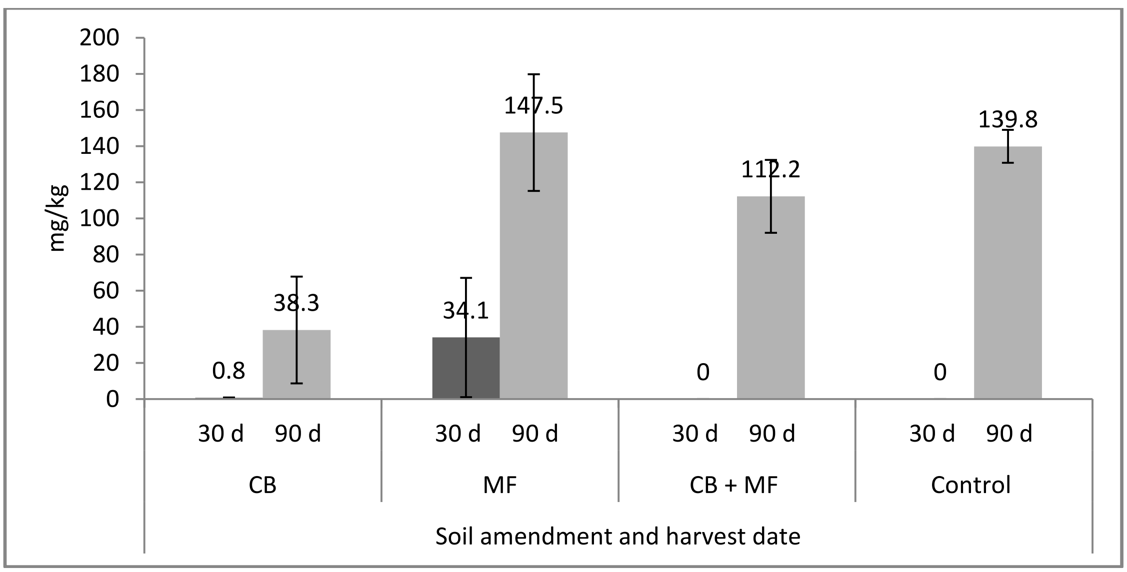

Figure 14. Lead concentration in above-ground biomass, ryegrass treatment, 30 d and 90 d.

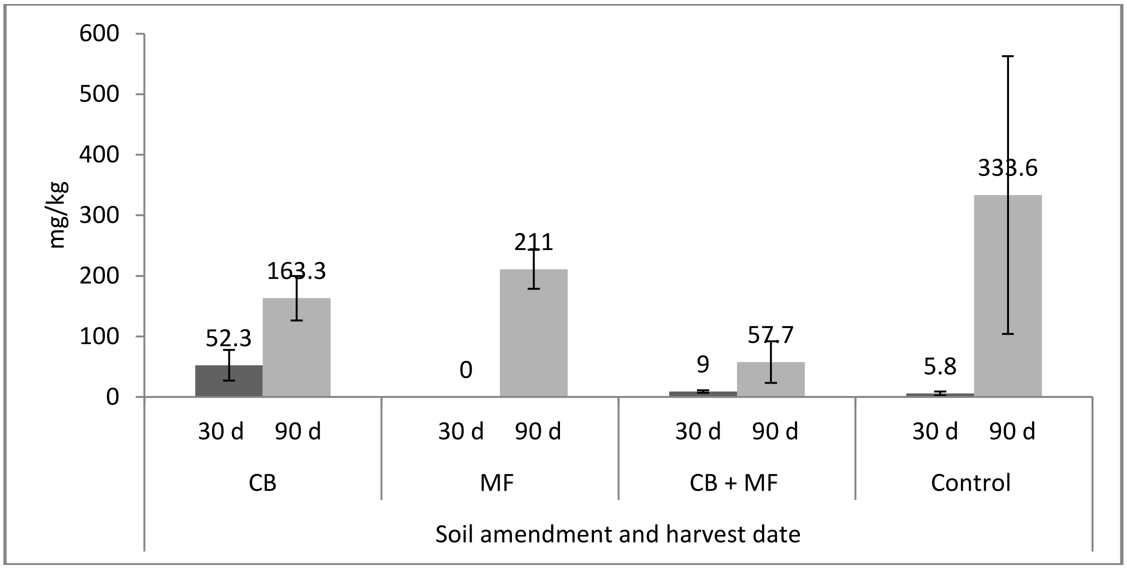

Figure 15. Lead concentration in above-ground biomass, red clover treatment, 30 d and 90 d.

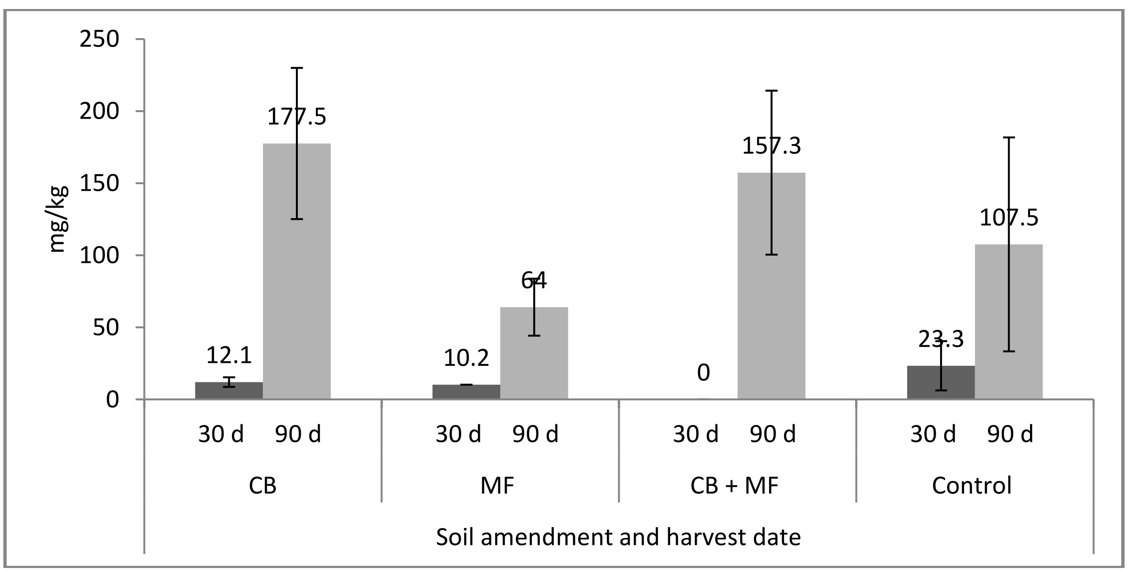

Figure 16. Lead concentration in above-ground biomass, sunflower treatment, 30 d and 90 d.

At 90 d, red clover (all soil treatments combined) concentrated the greatest quantity of Pb at 293.2 mg/kg while ryegrass contained the lowest quantity at 95.4 mg/kg (Table 12). Lead uptake by red clover (control) increased by 79% from 30 d to 90 d (5.8 to 333.6 mg/kg) ($p < 0.05$). In general, plant treatments concentrated more Pb from 30-90 growth, which is in contrast to Adesodun et al. (2010), who found that two genera of sunflower (*Tithonia diversifolia* and *Helianthus annus*) took up the greatest amount of shoot and root Pb from 0-30 days of growth.

On average, Pb root concentrations exceeded those of above-ground plant tissue concentration which supports the results of several other studies (Pichtel et al. 2000; Pichtel and Salt 1998; and Bricker et al. 2001). At 90 d sunflower roots (all soil treatments combined) concentrated the most Pb (656.6 mg/kg) while ryegrass concentrated the least (627.1 mg/kg) (Table 13). Padmavathiamma and Li (2007) suggested that sunflower is a promising species for removing Pb from various media due to its Pb tolerance and extensive root system. Ryegrass roots (control) concentrated the most Pb (977.4 mg/kg); ryegrass root Pb concentrations in other treatments ranged from 281.8 mg/kg (CB+MF) to 403.0 mg/kg (MF) ($p > 0.05$) (Table 14). Sunflower roots (control) contained 796.8 mg/kg Pb while sunflower roots in other treatments ranged from 147.4 mg/kg (CB+MF) to 699.3 mg/kg (MF) ($p < 0.05$) (Table 16). Red clover root Pb ranged from 174.9 mg/kg (CB) to 445.6 mg/kg ($p > 0.05$) (CB+MF) (Table 15).

Zinc

At 30 d ryegrass (all soil treatments combined) took up the most Zn at 1242.5 mg/kg while red clover accumulated the least at 903.5 mg/kg ($p < 0.05$) (Table 12).

Ryegrass (CB+MF) concentrated the most Zn at 1264.2 mg/kg and sunflower (CB+MF) concentrated the least at 304.4 mg/kg ($p < 0.05$) (Figs. 17,19). Zinc in soil and/or tissue can act as an antagonist to Cd and/or Pb toxicity to mycorrhizal fungi and plants (Gohre and Paszkowski, 2006). The high soil and tissue Zn concentrations may help explain the ability of several test plant species to tolerate the excessive concentrations of soil Cd and Pb (Tables 12,17-19).

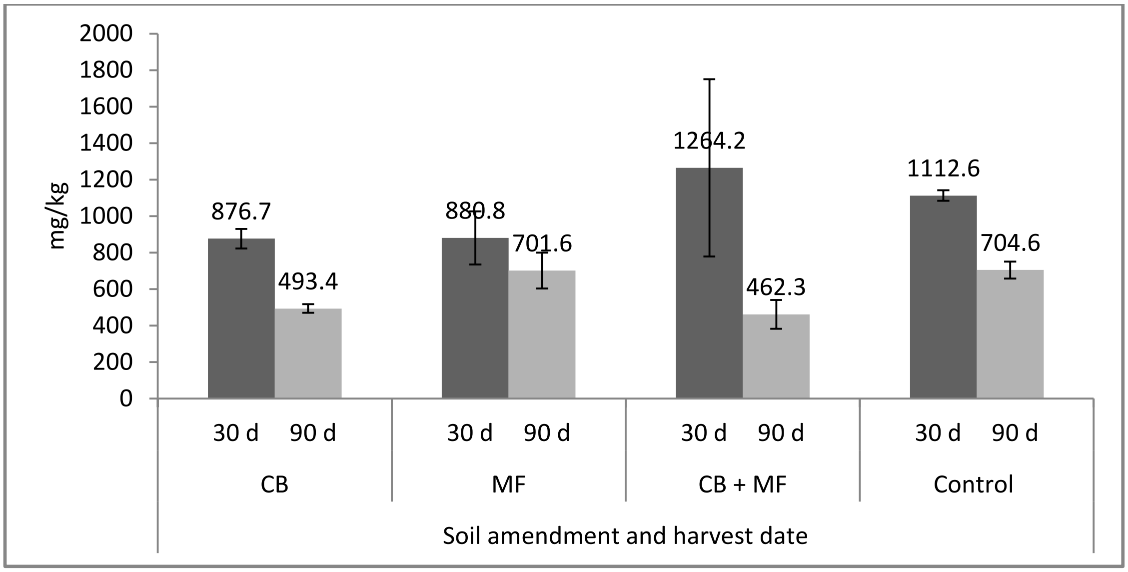

Figure 17. Zinc concentration in above-ground biomass, ryegrass treatment, 30 d and 90 d.

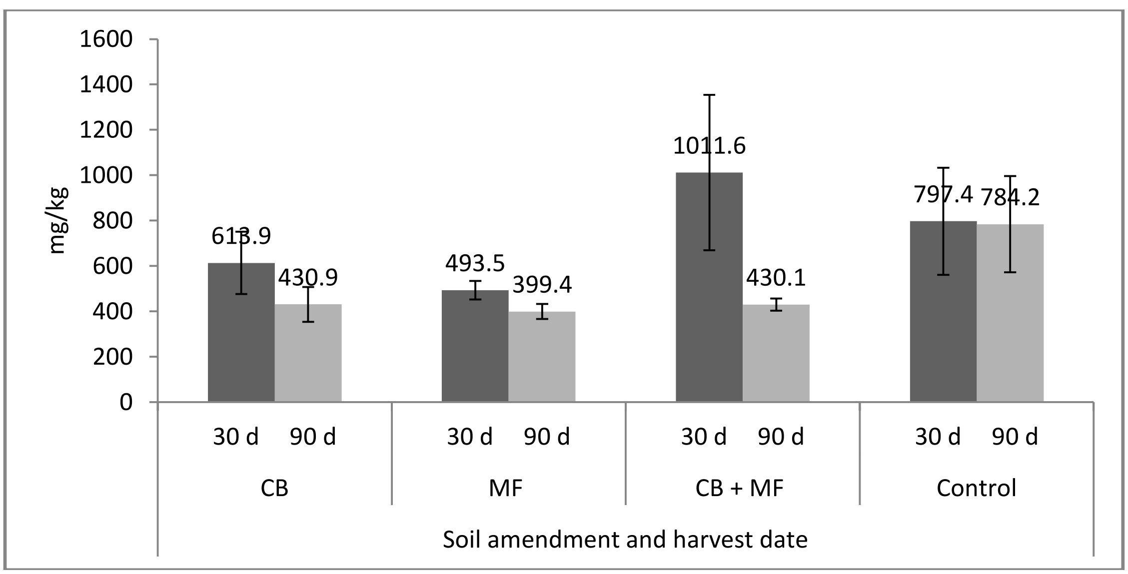

Figure 18. Zinc concentration in above-ground biomass, red clover treatment, 30 d and 90 d.

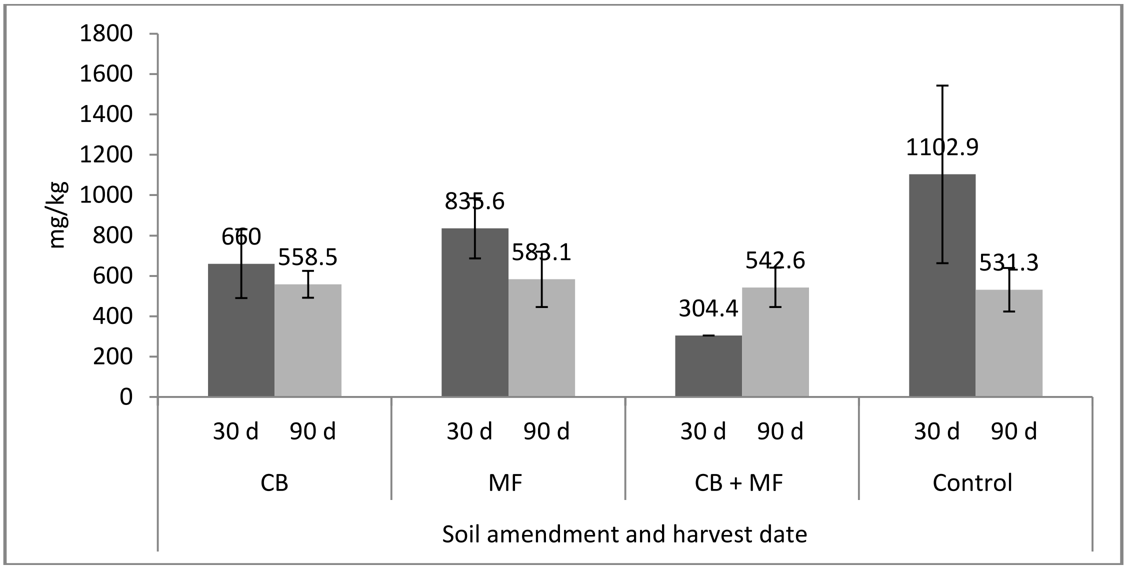

Figure 19. Zinc concentration in above-ground biomass, sunflower treatment, 30 d and 90 d.

At 90 d Zn uptake (all soil treatments combined) were similar for all plants, ranging from 567.3 mg/kg (ryegrass) to 675.3 mg/kg (red clover) (Table 12). Red clover (control) contained the most Zn at 784.2 mg/kg; red clover (MF) took up the least at 399.4 mg/kg. This difference is not signifigant ($p < 0.05$). Red clover (CB+MF) concentrated 1012 mg/kg Zn from 30 d, but by 90 d it contained 430.1 mg/kg; resulting in a 58% decrease (Fig. 18). This difference is significant ($p < 0.05$). Ryegrass (control) Zn concentrations remained steady from 30 d to 90 d (797.4 mg/kg and 784.2 mg/kg, respectively) (Fig. 17).

At 90 d ryegrass roots (all soil treatments combined) took up the most Zn (929.1 mg/kg), and sunflower roots took up the least Zn (332.6 mg/kg) (Table 13). This difference is significant ($p < 0.05$). Zn is generally >10 times more concentrated in plant roots than shoots, but in hyperaccumulating plants, shoot metal concentrations can be higher than root concentrations (Chaney et al. 1997). Smith and Bradshaw (1992) found that red fescue (*Festuca rubra*) was effective at stabilizing Zn from mine wastes (Gunawardana, 2011). Ryegrass roots (MF) concentrated 1100.7 mg/kg Zn and ryegrass (control) roots concentrated 1056.5 mg/kg (Table 14). Red clover roots (CB) took up the least Zn at 131.2 mg/kg but red clover roots (control) took up the most Zn at 1128 mg/kg (Table 15).

Field Study

At the field site, soil pH was 7.6, which was lower than the values measured in the greenhouse study (Table 2). Soil TOC was 3.8%, which was within the range of the greenhouse study soil. The site soil is highly variable in chemical and physical properties, due to a wide range of industrial and commercial uses for almost a century (Symbiont, 2009). Soluble NO_3^- and NH_4^+ measured 1.4 mg/kg and 3.1 mg/kg, respectively (Table 17) which are similar to those for greenhouse soil. Potassium concentrations were 1072.8 mg/kg, which are significantly ($p < 0.05$) higher than those for the greenhouse study (498.2 mg/kg) (all soil treatments combined) (Table 2). Average coverage percentage from the eight field plots, all of which were amended with CB, was 47% (data not tabulated). Ye et al. (2000) found that Italian ryegrass (*Lolium multiflorum*) experienced a higher percent coverage of test plots when grown on an amendment barrier of fly ash or combusted coal residue, as compared to bare soil.

Table 17. Selected soil chemical and physical properties.

	pH	TOC	NO_3^-	NH_4^+	K
		%	--------------------mg/kg------------------------		
Soil	7.6 ± 0.1	3.8 ± 1.2	1.4 ± 0.9	3.1 ± 2.4	1072.8 ± 52.3

	Extractable metals					
	Cd	Cu	Cr	Ni	Pb	Zn
	--mg/kg--					
Soil	6.3 ± 2.3	131.4 ± 17.7	15.4 ± 8.6	23.2 ± 3.7	55.0 ± 39.0	334.5 ± 78.5

Soil metals

Soil Cd concentration was 6.3 mg/kg, and soil Cu was 131.4 mg/kg (Table 17); both values are significantly less ($p < 0.05$) than that for the greenhouse study (Table 1). The Cd values are, however, still considered evidence of anthropogenic contamination (Kabata-Pendias, 2001). Soil Cr concentration was 15.4 mg/kg and soil Ni concentration was 23.2 mg/kg. Soil Pb was relatively low at 55.0 mg/kg; soil Zn measured 334.5 mg/kg (Table 17).

Plant tissue metals

Red clover did not become well established at the field plots. This may have been due, in part, to a significant drought occurring during part of the growing season. Furthermore, invasive species became established on many plots and may have also prevented red clover from becoming established.

Ryegrass tissue Cd was below detectable limits (Table 17). Tissue Cd concentrations from the 30 d and 90 d ryegrass (CB) of the greenhouse study were also low at 12.9 and 34.0 mg/kg, respectively (Fig. 1). Tissue Cu measured 124.5 mg/kg, which is comparable to the 90 d Cu value for ryegrass

(CB) in the greenhouse study (189.4 mg/kg) (Fig. 5). Tissue Cr measured 33.3 mg/kg (Table 18), which was less than tissue (CB) Cr 90 d greenhouse study at 75 mg/kg (Fig. 8). Tissue Ni was 72.8 mg/kg (Table 18); this value is greater than the 90 d ryegrass (CB) concentration from the greenhouse study at 46.8 mg/kg Ni (Fig. 14). Ryegrass (CB) tissue Pb (226.4 mg/kg) was significantly ($p < 0.05$) greater than that measured in the 30 d and 90 d greenhouse study (0.8 mg/kg and 38.3 mg/kg, respectively) (Fig. 12).

The lower concentrations of certain tissue metals from the field study may be due to the fact that those soils were heavily compacted and thus water movement was poor. Tissue Zn concentration was 303.5 mg/kg (Table 18), which was less than data for the 30 d and 90 d greenhouse study ryegrass (CB) (876.7 m/kg and 493.4 mg/kg, respectively) (Fig. 12). The extreme heterogeneity of the field soil may also have accounted for differences in ryegrass metal concentrations between the field study and greenhouse study. Regardless, however, the ryegrass experienced excellent cover of a slightly toxic and infertile soil material. It is suggested that this species be used for the revegetation of brownfield sites of similar chemical properties. Additionally, red clover showed a propensity for uptake of several toxic metals and should likewise be considered for revegetation and/or phytoremediation.

Table 18. Extractable metals in ryegrass tissue, field study.

Cd	Cu	Cr	Ni	Pb	Zn
----------	----------	----------mg/kg	----------	----------	----------
BDL*	124.5 ± 33.3	33.3 ± 17.8	72.8 ± 20.5	226.4 ± 217.9	303.5 ± 80.1

BDL* = Below detectable limit.

Conclusions

The three study plants (perennial ryegrass, *Lolium perenne*; red clover, *Trifolium pratense*; and sunflower, *Helianthus annuus*) all germinated and grew well on the soil from the brownfield site. This occurred despite the overall poor soil conditions (e.g., low organic matter content, low levels of fertility, high concentrations of several toxic metals, poor soil structure). Generally, perennial ryegrass and red clover are reported to be rather intolerant of metal-contaminated soils (Pichtel and Salt, 1998), but little to no signs of toxicity stress were indicated from any of the plant species. All species could, therefore, serve to revegetate brownfield sites in the US. It must be noted, however, that the compost amendment was valuable in increasing overall plant growth in the greenhouse and in field plots.

The three study plants varied in their uptake of heavy metals from the soil. Based on definitions provided by previous researchers, none of these can be considered hyperaccumulator species. Regardless, however, several species showed promise for metal uptake over the long term. This study has added to the understanding of phytoremediation and may suggest avenues of exploration for future soil-metal remediation.

The results of this study correlated in many respects to data from other phytoremediation experiments. Red clover produced relatively low biomass; however, it concentrated a greater quantity of metals and a greater variety of metals (Cu, Cr, Ni, and Pb from 30 d and 90 d) as compared to ryegrass and sunflower. Therefore, it may serve as a promising candidate for future work in phytoextraction. Metal uptake may be enhanced by the addition of synthetic or natural chelating agents.

Considering the accelerated rate of Cd and Pb by red clover as compared with ryegrass and sunflower, it is suggested that red clover possesses a mechanism(s) that allow translocation of metals into its biomass. These same mechanisms may provide tolerance against metal toxicity. In order for the maximal Cd to be taken up by red clover, phytoextraction processes should be carried out for multiple growing seasons. Red clover is also recommended as an accumulator for Pb-contaminated soils. Red clover and ryegrass also produced dense coverage, which helps prevent runoff and holds soil moisture more effectively. This dense soil coverage will contribute to organic matter additions to soil.

Compost application was associated with enhanced uptake of several metals by plants. Compost aided Cu uptake by sunflower, specifically from 30-90 d. Chromium uptake by ryegrass (CB) was significant ($p < 0.05$) from 30-90 d. In certain cases (e.g., ryegrass), the presence of compost may have restricted plant uptake of Pb. Lead in soil tends to be highly reactive, particularly at near-neutral pH. It is possible that soil Pb became tightly bound by soluble organic components of the compost.

Mycorrhizal fungi was associated with Cd and Pb uptake by ryegrass over 30-90 d. This amendment was also associated with enhanced Cr uptake by red clover. Mycorrhizal fungi had little impact on Pb uptake by red clover and sunflower. At this point, the benefits of mycorrhizal fungi on metal uptake by plants are conflicting. The ability of AMF to treat metal-enriched soil may depend on ensuring sufficient AMF biomass, i.e., it must occur in proportion to soil metal concentrations (Alori and Fawole, 2012). Small mushrooms were visible in some of the pots, indicating that while MF was thriving, its biomass may have been disproportionate to the moderate level of metal contamination.

Uptake of Cd, Cr, Pb and other metals was substantial in the roots of certain plant species. However, phytoextraction is optimal when metals of concern are translocated to above-ground biomass.

Given the poor fertility status (including low organic matter levels) of many brownfield sites, it is strongly recommended that revegetation efforts include application of an organic amendment. Many such amendments have the added benefit of providing natural chelating compounds, which may enhance metal uptake by the plant.

Although each brownfield site is unique and requires extensive and site-specific remediation plans, studies such as the current one can add to our understanding of how metals species behave in soil and what plant species/soil amendments work together to remove the greatest amount of metal contaminants in sustainable ways. It is also important to note that the unique nature of each brownfield can result in differing contaminant concentrations throughout one site, so plant/soil treatments that may be successful at one end of a site may not be suitable at another end of the same site. This is evident when considering the differences in plant metal uptake and other soil parameters between the field study and greenhouse study. Also, the fact that red clover did not survive from the field study but thrived in the greenhouse indicates that natural conditions are unpredictable. The conditions inside the do not necessarily reflect the environment that plants outside of human control may experience.

Suggestions for Future Research

The research site has experienced a long history of commercial and industrial use. As a result, soil chemical and physical properties have been highly variable. In order to better control such variability, future research should involve establishment of phytoremediation plots throughout the entire 5.4-acre site. The resultant data would yield a better understanding of site remediation processes.

Measurement of additional contaminants (e.g., hydrocarbons) and physical properties (saturated hydraulic conductivity, bulk density, structure) in the test soil would help determine whether these substances may have played a role in affecting plant growth and/or uptake of metals. Attempts should be made to correlate concentrations of specific hydrocarbons (e.g., naphthalene) or general TPH (total petroleum hydrocarbons) with overall plant health and with metal uptake.

Studies using other grasses (*Lolium*, *Agrostis*, *Poa*, etc.) and legumes (*Lespedeza*, *Vicia*) for site colonization and metal uptake are necessary. Likewise, other plant types should be assessed at the site. While the field study plots of the present study were limited to one location, the set up of plots at various and distant locations from each other on this brownfield site would yield a better understanding of the variety of organic and inorganic toxins, their concentrations, and how these toxins affect specific plant species. It may also be helpful to take inventory of the plants that grow wild (along with their density and percent coverage) on the site and determine whether any are taking up metals from the soil.

It is possible that the metals found in plant tissue, specifically Pb, could have been the result of genetic variation within the species; some varieties of red clover or ryegrass may have possessed genetic tolerance for passive Pb uptake while others may not have similar tolerance. Further research could involve growing 10-20 varieties of each species to identify the most metal-tolerant plants that could concentrate the most metal.

Additional study is needed to determine whether test plants would show signs of toxicity after 90 days of growth in the test soil. Furthermore, repeated croppings over a period of 3-4 years would demonstrate the ability of the test crops to create a self-sustaining ecosystem on the site.

It is hypothesized that the variety of metals uptake by red clover could be further enhanced by application of natural or synthetic chelating agents. Such work would be combined with extensive surveys of soil microbiological parameters, as chelating agents have been shown to result in stunted plant/root growth and to harm soil organisms (Romkens et al., 2002). Studies are also needed to characterize the possible metal-solubilizing compounds that red clover roots secrete.

The overall effect of mycorrhizal fungi in association with plant roots is an area that needs greater study in order to better understand the sometimes conflicting results for this treatment. It is possible that mycorrhizal fungi may have secreted amino acids or natural chelates into the rhizosphere that bind with essential plant nutrient metals which could then more easily be transported through xylem plant tissue. Mycorrhizal fungi also increased Cd concentrations in sunflower; further research is needed to provide insights into the mechanisms of action.

Study Limitations

The reported study demonstrated that certain plant and soil treatment combinations resulted in metal extraction or immobilization from soil; however, certain limitations must be taken into consideration by researchers pursuing future work on this topic. The field study took place in Indiana which is part of the Great Lakes region of the US. Its climate is defined as humid continental, with an average of 40 in. of rainfall per year (USGS, 2009). Indiana can experience significant fluctuations of day-to-day weather, and even though many plant species are adaptable to differing conditions, those employed for remediation must be cultivated under optimal temperature and moisture regimes in order to maximize metal uptake. Incidentally, this did not occur in the field study because of a severe summer drought which resulted in several days of 90-100^{o}F daytime temperatures. This weather extreme affected growth of red clover while ryegrass was able to survive. The greenhouse study was highly controlled in terms of climate and air quality. The tested plants received light 24/7 and temperature and humidity were kept constant.

Field studies may require substantial application of herbicides and insecticides in order to control pests; alternatively, many hours of manually removing weeds and pest insects is required. It is also essential to ensure that phytoremediation plants do not create invasive species management problems.

The brownfield soil was considered to be only slightly contaminated, which may account for why test plants experienced little or no toxicity effects. The methods utilized and the subsequent success experienced from the greenhouse study may not be observed with soil categorized from severely metalliferous sites, such as those listed on the National Priority List (NPL).

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